RESEARCHREPORT

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ANIMAL WELFARE

Title: Evaluation of a novel means to euthanize piglets – **NPB #10-123**

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Date Submitted: October 18, 2012

Industry Summary

Electromagnetic (EM) energy transmitting devises that operate in the microwave frequency have been approved by the American Veterinary Medical Association for the specific use in euthanasia of laboratory mice and rats and have been also applied to chickens. Units utilized for this method of euthanasia focus all EM (microwave) energy to the head of the animal targeting the brain for very rapid elevation of brain temperature. This increase in brain temperature results in denaturation of neural connections and very rapidly incapacitates all brain function; including any perception of pain. With this in mind, EM energy poses a possibility for the development of a new tool to be utilized when it becomes necessary to euthanize piglets. The primary objective of this project was to test the utilization of EM energy as a potential means of euthanizing piglets on the farm. Experiment 1 assessed whether or not EM energy could bring anesthetized piglets to death and Experiment 2 evaluated the consciousness, unconsciousness, post-EM energy exposure (EMEE), and death of piglets.

In Experiment 1, six anesthetized piglets were exposed to 40 s of EMEE. Prior to EMEE, heart rate, respiration rate, head surface temperature, and internal body temperature were recorded. Following EMEE, heart rate, respiration rate, head surface temperature, and mid-brain temperature were recorded. Prior to EMEE, internal body temperature was 37.7ºC and following EMEE intracranial temperature was 62.8ºC. Head surface temperature was higher ($P < 0.01$) after EMEE (32.7 vs. 69.2ºC, respectively). Moreover, respiration stopped in each piglet immediately following EMEE. Unassisted death occurred after heart rate ceased within 4.8 min after EMEE application in five of the six piglets.

Treatments in Experiment 2 included EMEE for 3, 6, or 9 s administered to 8 piglets per treatment (24 total). Electroencephalogram (EEG) and electrocardiogram were recorded during each stage of consciousness. Additionally, head surface and internal body temperatures were recorded before and after EMEE. State of consciousness and treatment interaction affected ($P < 0.01$) EEG amplitude and power. State of consciousness affected ($P < 0.01$) EEG frequency. Sex affected ($P < 0.01$) EEG amplitude. Further, piglets exposed to 9 s EMEE had higher ($P < 0.01$) body temperature compared with piglets exposed to 3 and 6 s EMEE (40.6 vs. 39.1 and 39.6ºC, respectively). Piglets exposed to 9 s EMEE had higher post EMEE head surface temperature than piglets exposed for 3 s (39.7 vs. 34.9ºC, respectively). Moreover, heart rate was reduced ($P < 0.01$) from conscious to unconscious to after EMEE applications.

This research provides insight to the possibility of developing new techniques as well as the potential for improving current methods of euthanizing piglets. Our study is unique in its use of EEG analysis of piglets during consciousness, unconsciousness, and death. Our observations and analysis have aided in the establishment of baseline EEG values for piglets. With further research in this area and an increase in sample size, there is a possibility to improve on-farm husbandry and euthanasia techniques with regard to animal welfare. With further research, a new technique

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could be available to humanely euthanize piglets. Experiment 1 demonstrated that EM energy results in the death of a piglet and with modifications of our equipment and EEG data, we will be able to better understand the effects of EM energy on the sensibility of piglets. Based on the EEG data from this study, it would be recommended that future trials utilizing the same amount of EM energy expose the piglet in excess of six seconds. Since piglets were under a surgical plan of anesthesia when exposed to EM energy, it is still unclear as to the actual effect EM energy has on the sensibility of piglets and potential for perception of pain during application. Future research is necessary to provide a better understanding regarding attaining pain insensibility. Once the temperature critical limit for brain inactivation (establishment of pain insensibility) has been determined, the duration of electromagnetic energy exposure is irrelevant. Energy could be administered for sufficient duration to achieve complete brain denaturation and physical death.

Keywords
electroencephalogram, electromagnetic, euthanasia, humane, piglets

Scientific Abstract

This research was conducted to evaluate a novel means to euthanize piglets utilizing electromagnetic (EM) energy administered at 2,450 MHz power. Experiment 1 assessed whether or not EM energy could bring piglets to death and Experiment 2 evaluated the consciousness, unconsciousness, post-EM energy exposure (EMEE), and death of piglets. In Experiment 1, six anesthetized piglets were exposed to 40 s of EMEE. Prior to EMEE, heart rate, respiration rate, head surface temperature, and internal body temperature were recorded. Following EMEE, heart rate, respiration rate, head surface temperature, and intracranial temperature were recorded. Prior to EMEE, internal body temperature was 37.7ºC and following EMEE intracranial temperature was 62.8ºC. Head surface temperature was higher ($P < 0.01$) after EMEE (32.7 vs. 69.2ºC, respectively). Moreover, respiration rate was reduced to zero in each piglet immediately following EMEE. Immediately after EMEE, heart rate slightly ($P = 0.86$) decreased (133 bpm prior vs. 129 bpm post EMEE). Unassisted death occurred after heart rate ceased within 4.8 min after EMEE application in five of the six piglets. Treatments in Experiment 2 included EMEE for 3, 6, or 9 s. In Experiment 2, electroencephalogram (EEG) and electrocardiogram were recorded during the states of consciousness mentioned above. Additionally, head surface and internal body temperatures were recorded before and after EMEE. State of consciousness and treatment interaction affected ($P < 0.01$) EEG amplitude and power. State of consciousness affected ($P < 0.01$) EEG frequency. Sex affected ($P < 0.01$) EEG amplitude. EMEE and treatment interaction also affected internal body and head surface temperatures ($P < 0.01$). Piglets exposed to 9 s EMEE had higher body temperature compared with piglets exposed to 3 and 6 s EMEE (40.6 vs. 39.1 and 39.6ºC, respectively). In addition, piglets exposed to 9 s EMEE had higher post EMEE head surface temperature than piglets exposed to 3 s EMEE (39.7 vs. 34.9ºC, respectively). Moreover, heart rate was reduced ($P < 0.01$) from conscious to unconscious to after EMEE applications; however, not immediately after euthanasia (172, 133, 88, and 129 bpm, respectively). In conclusion, three seconds is not enough time to denature brain tissue to a point that potentially eliminates the perception of pain. Thus, more than six seconds of EM energy is needed. After reaching insensibility, the EM energy exposure could be carried to the point of complete brain denaturation and physical death.

Introduction

As the global pork industry continues to grow, technologies evolving euthanasia research will be increasingly important regarding animal welfare. On farm euthanasia of piglets is an inevitable and necessary means to alleviate animal suffering due to illness, injury, or other circumstances. Approved methods for euthanizing piglets must address concerns for animal welfare and/or worker safety. Development of a new method of euthanasia could have positive implications for pork producers as current methods of piglet euthanasia have rapidly become a point of controversy. Consequently, an opportunity exists for the pork industry to further study current and novel means of euthanasia in all aspects of production.

Electromagnetic (EM) energy poses a possibility for the development of a new tool to be utilized when euthanizing piglets. Instruments with microwaves are approved and have been specifically designed for use in euthanasia of laboratory mice and rats. Units utilized for this method of euthanasia direct all EM energy to the head of the animal essentially targeting the brain (AVMA, 2007).

With that in mind, the primary objective of this project was to test the utilization of EM energy as a means of euthanizing piglets on the farm. This study will help determine if EM energy can be utilized as an acceptable means of euthanizing piglets. The present study will assess whether or not the EM energy can bring piglets to death (Experiment 1). In addition, quantitative EEG (qEEG) technology will be used to characterize the conscious, unconscious, post-stun, and death states in piglets after administration of EM energy directly to the cranium for 3, 6, and 9 seconds. These data will provide an indication of brain tissue thermal denaturation across the three lengths of EMEE and provide insight regarding the point denaturation may be sufficient to cease neural perception of pain (Experiment 2).

Original Objective

a) Determine the feasibility to render a piglet dead in haste (2 to 4 seconds) by means of administering electromagnetic energy (5, 10, and 15 kilowatts for 2 and 4 sec) to the head, cessation of heartbeat, and cessation of respiration.

Materials & Methods

All methods and procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee at North Dakota State University (#A10042).

EM Unit Development: AMTek Microwaves (Cedar Rapids, IA) was consigned to develop a 2,450 MHz Electromagnetic energy device as a means for piglet euthanasia. The unit was to be contrived based on unpublished research using a 915 MHz transmitter and a ridged EM waveguide. Conventional applications of EM energy administer energy in an unfocused manner to avoid accumulation of hotspots in the warming of the target. The waveguide used for the present research project was designed specifically focus delivery of the EM energy to the target tissue (cerebral cortex). A single horn waveguide had been previously tested (unpublished data) on larger hogs. Personal communication with Dr. John Osepchuk, a renowned microwave power engineer (Full Spectrum Consulting), regarding the potential for a hot spot at the junction of two microwave signals led us to a new design using a dual transmitter power unit applicator with two ridged horns set at 90 degrees to each other. The application of EM energy from two opposing sides would exploit the hypothesis that more rapid heating in the microwave range would occur at the confluence of the energy fields.

The two microwave generators provided were developed especially for NDSU from a version currently supporting the utility industry (Figure 1). Each of the two microwave power units contains one air-cooled 1.45 kW magnetron which develops 1,450 watts of continuous microwave energy at the 2,450 MHz frequency. By using two power units, the combined total energy available is 2,900 watts. Each generator is equipped with a separate ridged waveguide scaled down from the successful 915 MHz horn previously mentioned. A countertop microwave oven was modified to accept the body of a 15 lb piglet and the two ridged waveguide horns were mounted in the roof of the unit at a 90 degree angle with the broad walls adjacent to each other. The on/off switch and timer of the countertop microwave were wired to the on/off circuits of the two independent generators. This allowed the countertop timer to start both generators simultaneously. Upon pressing “start”, the filament requires a short duration to warm and the high voltage generation of microwave energy. Titration experiments (unpublished data) revealed the warm-up time for the filament was between 1 and 1.5 seconds. During this interval, spurious wavelength signals are generated, but little power at 2,450 MHz is produced. All tests were run assuming a 1 second delay and times were adjusted accordingly.

Experiment 1

Animals. Six piglets (4.3 ± 0.66 kg of BW) were obtained from a commercial swine operation. Before the start of the experiment, piglets were transported to the North Dakota State University Animal Nutrition and Physiology Center.

Treatment. The experiment utilized an electromagnetic containment box (ECB) designed to apply EM energy to the left and right side of the head. All piglets were exposed to 40 seconds of EM energy treatment simultaneously to both sides of the head targeting the piglet’s cerebral cortex. The 40 seconds application time was selected based on a titration experiment that utilized deceased piglets (Fig. 2) whereby different durations of EM energy were tested and it was determined that 40 seconds was the optimal amount of time to achieve 30ºC increase in midbrain temperature.

Data Collection. As piglets were manually immobilized, initial surface temperature of piglet head was recorded utilizing a thermal imaging camera (Fluke Ti 25; Fluke Corporation, Everett, WA, USA) and initial internal body temperature was recorded using a thermoscan ear thermometer (Model IRT 4520; BraunTM, San Francisco, CA, USA). Additionally, initial respiration and heart rate were manually calculated and recorded. Prior to EM energy application, piglets were administered a combination of Telazol and Xylazine (0.18 mL/kg BW) to undergo a surgical plane of anesthesia. After completely anesthetized (pupils fixed and dilated; no pain response), piglets were placed in a full body cloth restraining apparatus to prevent movement in the ECB during application of EM energy to the head of the piglet. During the 40 seconds of EM cycle, movements and visual assessments of the piglets were recorded. Upon cessation of the EM cycle, piglets were removed from the ECB and post temperatures (head surface temperature and midbrain temperature), heart rate, respiration rate, and time of death were recorded. Midbrain temperature was recorded utilizing a digital thermometer (Model HH801B; OMEGA Engineering Inc., Stamford, CT, USA).

Statistical Analysis. Data were analyzed as a paired analysis using generalized least squares (PROC MIXED, SAS Institute, Cary, NC). The model included EM energy application (pre vs. post), sex, and their interaction as fixed effect and piglet ID as random effect. The interaction between EM energy application and sex was clearly non-significant (P > 0.30); thus removed from the final model.

Experiment 2

Animals. To achieve the objective of this experiment, 24 piglets (4.0 ± 0.28 kg of BW) were obtained from a commercial swine operation. Before the start of the experiment, piglets were transported to the North Dakota State University Animal Nutrition and Physiology Center, where they were sorted by BW and to sex, and then randomly allotted 1 of 3 treatment groups.

Treatments. The experiment utilized an electromagnetic euthanasia containment box (ECB) to apply EM energy simultaneously to both left and right side of the head of each piglet. The 3 experimental treatments were application of EM energy for 3 (Treatment 1), 6 (Treatment 2), or 9 (Treatment 3) seconds. These three times were selected based on previous unpublished research from our group that indicated exposure to EM energy for approximately five seconds would bring market hogs to insensibility (pupils fixed and dilated; no pain response to nose prick).

Data Collection. Piglets were weighed individually in order to sort them by weight and to calculate proper dosage of anesthesia protocol. As piglets were manually immobilized, initial surface temperature of piglet head was recorded utilizing a thermal imaging camera (Fluke Ti 25; Fluke Corporation, Everett, WA, USA), initial body temperature was recorded using a thermoscan ear thermometer (Model IRT 4520; BraunTM, San Francisco, CA, USA), and conscious EEG and electrocardiogram (EKG) were recorded utilizing Trackit TM Sleep Walker recorder (Lifelines Neurodiagnostic Systems, Inc., Troy, IL). After 5 minutes of conscious EEG and EKG recording, piglets were administered a combination of Xylazine and Telazol (0.05 mL/kg BW) to undergo a surgical plane of anesthesia. Piglets were considered anesthetized and unconscious as pupils became fixed and dilated; 5 more minutes of EEG and EKG were recorded under this state. Following anesthetized EEG and EKG recording, piglets were placed in a full body cloth restraining apparatus to prevent movement in the ECB during application of EM energy to the brain of the piglet. Following EM energy application, piglet brain activity and heart rate were further recorded. Additionally, surface temperature of piglet head and body temperature were recorded after EM energy application. After 5 minutes of EEG and EKG recording post application of EM energy, piglets were chemically euthanized utilizing Euthasol (1 mL/4.53 kg BW) intracardially. Two minutes of EEG and EKG recording were obtained after Euthasol application. For EEG/EKG recording, four channels were used as L1, L2, R1, and R2 with a reference and a ground electrode (Fig. 3).

EEG Analysis. Brain activity was recorded utilizing four channels, a reference, and a ground electrode connected to the Trackit TM Sleep Walker recorder (Lifelines Neurodiagnostic Systems, Inc., Troy, IL). After EEG was obtained in the four states of consciousness (conscious, unconscious, post EM energy application, and cease of respiration) raw EEG traces were analyzed using the Magic Marker (Persyst Insight II; Persyst Development Corporation, Prescott, AZ) software. Raw EEG traces were observed and two seconds artifact free epochs were selected and processed for fast Fourier transform (FFT) spectral analysis with reference to active electrodes according to a L1-Ref; L2-Ref; R1-Ref; and R2-Ref montage. The three parameter obtained from Magic Marker were amplitude (aEEG), frequency (FFT-Edge), and power (FFT-Power). Values obtained from each electrode were averaged to give one numerical value for each parameter. Amplitude, reported in microvolts (μV), was obtained with a sampling rate of 64 Hz and filters constant at 0.16 seconds, notch of 60 Hz, and high filter off. Frequency, reported in hertz (Hz), was obtained with the bar option and calculated with 128 Hz sampling rate, 256 points per window, 2 window duration, 2 epoch duration, and 0.5 Hz frequency resolution; the frequency band was set from 0 to 45 Hz with 95% edge. Last, power, reported in microvolts squared (μV2), was also obtained with the bar option and similar calculations as frequency were used; the asymmetry spectrum was between 0 to 32 Hz.

Statistical Analysis. Data were analyzed using generalized least squares (PROC MIXED, SAS Institute, Cary, NC). The model included treatment, state of consciousness, sex, and the respective interactions as fixed effect. State of consciousness was used as repeated measures with piglet ID(treatment) as the subject. The correlation structure over state of consciousness was modeled to minimize Akaike's Information Criteria (Littell et al., 2006). Interactions that were clearly non-significant (P > 0.30) were removed from the model. In addition, linear and quadratic contrasts within treatment were calculated.

Results & Discussion

Experiment 1

There were no effects of treatment interaction with sex and the sex of piglets alone for any of the response variables ($P \geq 0.16$). Therefore, only effects of treatment (EM energy application) are presented.

Heart Rate. Heart rate was numerically affected ($P = 0.86$) immediately after exposure to EM energy (133 vs. 129 bpm, respectively). However, 4.8 min (± 1.79 min) following the application of EM energy five out of six piglets had ceased heart rate. For these five piglets, heart rate cessation ranged from 3 to 7 min post EM energy. One piglet was chemically euthanized (Euthasol) because its heart remained beating (although steadily declining) after the predetermined threshold of 10 minutes. It is important to note that all piglets ceased respiration upon removal from the ECB (see below).

Respiration Rate. Respiration rate was drastically reduced ($P < 0.01$) to zero in each piglet immediately following exposure to EM energy; thus, EM energy had a direct effect on the respiration rates of each piglet. Prior to EM application, piglets' respiration rate was 49 rpm. Close et al. (1996) stated that respiration rate is one of the most important aspects in the recognition of death. Also, Grandin (2001) stated that to provide the best possible animal welfare, a stunning (euthanasia) method must instantly render an animal completely insensible to pain and the animal must not return to sensibility. Therefore, cease of respiration observed immediately after exposure to EM energy followed by consequently stop of heart rate supports the hypothesis that EM energy can result in a successful euthanasia of piglets. The question remains as to whether or not pain is experienced in route to cease of respiration.

Temperature. Treatment affected ($P < 0.01$) average head surface temperature (Fig. 4). Average head surface temperature measurements were 32.7 prior versus 69.2ºC after EMEE.

Prior to exposure to EM energy, mean internal body temperature was 37.7 ºC. Following exposure to EM energy, mean intracranial temperature was 62.8 ºC. Gao et al. (2010), Fagan (1997), and Branden and Tooze (1998) reported that temperatures exceeding 41°C will cause proteolysis which would lead to nervous cells denaturation and loss of function. Further, it can be concluded that as nervous cells denature and lose function, the ability to perceive pain is eliminated.

Experiment 2

EEG parameters. The three way interaction among treatment, state of consciousness, and sex and the two way interactions between treatment and sex and state of consciousness and sex did not affect any of the three EEG parameters measured ($P \geq 0.10$). Therefore, only the interaction between treatment and state of consciousness and the main effects of treatment, state of consciousness, and sex will be mentioned below.

Amplitude. Amplitude is an indication of the amount of electrical energy measured from active neurons and synapses. Amplitude (aEEG) was affected ($P < 0.01$) by state of consciousness interaction with treatment (Fig. 5). Amplitude EEG values for each treatment in the conscious state were 21.04, 22.47, and 21.25 µV for treatments 1, 2, and 3, respectively. Amplitude EEG values for each treatment in the unconscious state were 42.71, 48.70, and 44.87 µV for treatments 1, 2, and 3, respectively. Amplitude EEG values for each treatment following exposure to EM energy were 41.89, 26.66, and 20.59 µV for treatments 1, 2, and 3, respectively. Finally, aEEG values for each treatment after cease of respiration (chemical euthanasia) were 2.66, 2.53, and 3.63 µV for treatments 1, 2, and 3, respectively. EEG Amplitude was much lower ($P < 0.01$) in treatment 3 compared with treatment 1 following EM energy application. This indicates that time of exposure should confidently exceed six seconds.

Within each treatment, from the conscious to unconscious (anesthetized) states, piglets had an aEEG increase of 52%. The increase in amplitude recognized here is supported by other literature (Voss and Sleigh, 2007 and Bo et al., 2003) and can be explained by the neurons becoming more synchronized as body functions are limited during unconsciousness. Additionally, from the unconscious state to after the EM exposure there as a 37% reduction in aEEG, which demonstrates that neurons were being affected by the EM energy. Moreover, following cease of respiration after chemical euthanasia aEEG was 90% lower compared with aEEG following exposure to EM energy.

With reference to treatment 1 (3 s EMEE application), aEEG values increased from the conscious to unconscious state. Further, aEEG values were lower during the conscious state than the state immediately following exposure to EM energy. Amplitude EEG values were similar from the unconscious state to the state immediately following exposure to EM energy. However, there was a decrease in aEEG values from both the unconscious state and the state immediately following exposure to EM energy to the state following cease of respiration.

With respect to treatment 2 (6 s EMEE application), aEEG values increased from the conscious to the unconscious state. Additionally, aEEG values tended to be lower when comparing values obtained from the conscious state to values obtained from the state immediately following exposure to EM energy. Amplitude EEG values decreased from the unconscious state to the state immediately following exposure to EM energy. Amplitude EEG values obtained from the state following cease of respiration were much lower than the other three states of consciousness.

With reference to treatment 3 (9 s EMEE application), there was also an increase in aEEG values from the conscious to unconscious state. Values obtained from the conscious state and the state immediately following exposure to

EM energy was similar. Amplitude EEG values obtained following cease of respiration were lower than values obtained in the other three states of consciousness.

Moreover, aEEG values averaged across all levels of consciousness were greater ($P = 0.03$) in female piglets compared with male piglets (26.71 vs. 23.12 µV, respectively). This difference can be seen in Figure 6. This observation supports findings from Golgeli et al. (1999) which indicate that aEEG values obtained from males and females differ within specific channels.

Frequency. Frequency relates to the number of cycles or waves per second of EEG recording. Frequency values were minimally affected by the interaction between treatment and state of consciousness and the main effects of sex and treatment ($P \geq 0.24$). However, frequency EEG (FFT-Edge) values differ ($P < 0.01$) among the four states of consciousness (Fig. 7). Frequency EEG values for consciousness, unconsciousness, the state following EM energy exposure, and the state following cease of respiration were 32.96, 13.78, 11.41, and 34.31 Hz, respectively. FFT-Edge values decreased 58% from the conscious to unconscious state. Similar to amplitude of EEG, the observation that frequency decreases as piglets' transition from the conscious to unconscious state is supported by literature that reports finding of slower waves of higher amplitude from conscious to unconscious states (Voss and Sleigh, 2007 and Bo et al., 2003). As piglets were exposed to EM energy, frequency of EEG remained in an acceptable range for unconsciousness (Kaiser, 2006). This indicates that EM energy did not bring piglets out of the unconscious state and EM energy does not affect the brain in terms of frequency when exposed for less than or equal to 9 seconds. From the state of consciousness following exposure to EM energy to the state of consciousness following cease of respiration there was a 67% increase in FFT-Edge values. As most EEG work is done in humans, it is difficult to explain an increase in frequency values post-death due to the fact that most human patients remain alive during the duration of individual studies.

Power. Power was calculated as the square of the EEG magnitude. EEG magnitude was calculated from the average peak to base amplitude obtained from the EEG signal across the sampling duration 9formerly described as epoch). Sex had only a slight effect ($P = 0.24$) on power. However, the interaction between treatment and state of consciousness affected FFT-Power ($P < 0.01$). This interaction is represented in Figure 8. FFT-Power values for each treatment in the conscious state were 65.45, 80.49, and 68.31 μV^2 for treatments 1, 2, and 3, respectively. FFT-Power values for each treatment in the unconscious state were 310.25, 413.92, and 303.68 μV^2 for treatments 1, 2, and 3, respectively. FFT-Power values for each treatment following exposure to EM energy were 312.10, 174.42, and 110.47 μV^2 for treatments 1, 2, and 3, respectively. Finally, FFT-Power values for each treatment after cease of respiration were 0.91, 0.00, and 2.38 μV^2 for treatments 1, 2, and 3, respectively. Power values were much lower ($P < 0.01$) in treatment 3 compared with treatment 1 following EM energy application. Additionally, a tendency ($P = 0.08$) was observed for FFT-Power values to be lower in treatment 2 than in treatment 1. These FFT-Power results are similar to the EEG amplitude values. As a result of FFT-Power values not being affected by EM energy during treatment 1, it can be assumed that three seconds of EM energy exposure does not impact qEEG values; thus, exposure time needs to be greater than three seconds and more evaluation is necessary to identify the ideal time.

Moreover, from the conscious to unconscious, piglets in each treatment had a great increase (79%) in EEG power. Additionally, from the unconscious state to after the EM exposure there as a 42% reduction in FFT-Power. Moreover, following cease of respiration EEG power values decreased 100% from the time after EM energy exposure.

Heart Rate. The three way interaction among treatment, state of consciousness, and sex, the two way interactions between treatment and sex, treatment and state of consciousness, and state of consciousness and sex, and the main effect of treatment did not affect heart rate ($P \geq 0.10$). Therefore, only the main effects of state of consciousness and sex will be addressed.

As can be seen in Figure 9, heart rate decreased ($P < 0.01$) from the conscious to unconscious state (175 vs. 133 bpm, respectively). Furthermore, heart rate also decreased ($P < 0.01$) from the unconscious state to the state following exposure to EM energy (133 vs. 84 bpm, respectively). Immediately after chemical euthanasia (Euthasol), heart rate increased ($P < 0.05$; 88 vs. 129 bpm, respectively) prior to its complete stop. As we recognize the increase in heart rate it can be explained by the heart's will to survive that is noted in other literature prior to death (Marple et al., 1974).

Moreover, heart rate values tended ($P = 0.08$) to be greater in male piglets compared with female piglets (142 vs. 118 bpm, respectively). This difference can be seen in Figure 10.

Temperature. The three way interaction among treatment, state of consciousness, and sex, the two way interactions between treatment and sex and state of consciousness and sex, and the main effect of sex did not affect piglets' body nor average head surface temperatures ($P \geq 0.10$). Therefore, only the interaction between treatment and state of consciousness are mentioned.

Body temperature was effected ($P < 0.01$) by the interaction between treatment and application of EM energy (Fig. 11). Pre application of EM energy, body temperature did not differ ($P \geq 0.28$) among treatments; however, after EM energy application body temperature was higher ($P < 0.01$) in piglets in treatment 3 compared with piglets in treatment 1 (40.6 vs. 39.1 ºC, respectively).

Furthermore, the interaction between treatment and application of EM energy also had an effect ($P < 0.01$) on average head surface temperature (Fig. 12). Prior the application of EM energy, average surface head temperature did not differ ($P \geq 0.93$) among treatments; however, post EM energy application the head surface temperature of piglets in treatment 3 was higher ($P < 0.01$) when compared with the post EM energy application head surface temperature of piglets in treatment 1 (39.7 vs. 34.9 ºC, respectively).

Temperature findings indicate that as exposure time to EM energy increases, body temperature does as well. Treatment 3 reported a post EM energy body temperature of 40.6 ºC, which is just under the 41ºC threshold supported by literature leading to nervous tissue denaturation (Gao et al., 2010; Fagan, 1997; and Branden and Tooze, 1998). If systemic body temperature is elevated to 40.6 ºC, we can assume that the direct application of EM to the brain resulted in an even higher temperature directly to the brain.

Implications

This research provides further insight to the possibility of developing new and improving current methods of euthanizing piglets. Our study is unique in its qEEG analysis of piglets during consciousness, unconsciousness, and death. By utilizing 24 piglets, our observations and analysis have aided in the establishment of baseline qEEG values for piglets. With further research in this area and an increase in sample size, there is a possibility to improve on-farm husbandry and euthanasia techniques with regard to animal welfare.

Our observations of EM energy being utilized as a means to euthanize piglets leads us to believe that with further research, a new technique can be available to humanely euthanize piglets. Experiment 1 demonstrated that EM energy results in the death of a piglet and with modifications of our equipment and qEEG data, we will be able to better understand the effects of EM energy on the sensibility of piglets. Based on the qEEG data from this study, it would be recommended that future trials utilizing the same amount of EM energy to euthanize piglets expose piglet in excess of six seconds. Since piglets were exposed to EM energy during the state of unconsciousness, it is still unclear as to the actual effect EM energy has on the sensibility of piglets and potential for perception of pain during application.

We conclude from Experiment 2 that three seconds of EM application is not sufficient time to denature brain tissue to a point that eliminates perception of pain. Future research utilizing EM energy administered using the dual ridged waveguides using conventional microwave power (2,450 MHz) for durations equal to or exceeding six seconds will provide a better understanding regarding attaining pain insensibility. Once the critical limit for brain inactivation (establishment of pain insensibility) has been determined, the duration of electromagnetic energy exposure is irrelevant. Energy could be administered for sufficient duration to achieve complete brain denaturation and physical death.

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Figure 1. Dual generator electromagnetic unit utilizing two microwave power units, each containing one air-cooled 1.45 kW magnetron which develops 1,450 watts of continuous microwave energy at the 2,450 MHz frequency.

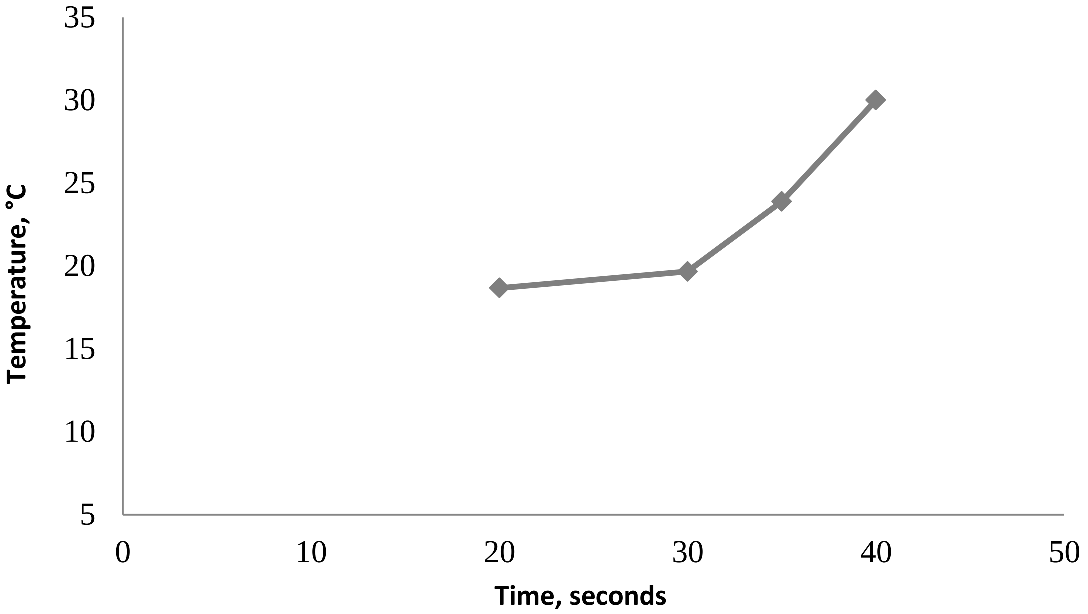

Figure 2. Effect of EM energy cycle on temperature change in the brain of deceased piglets.

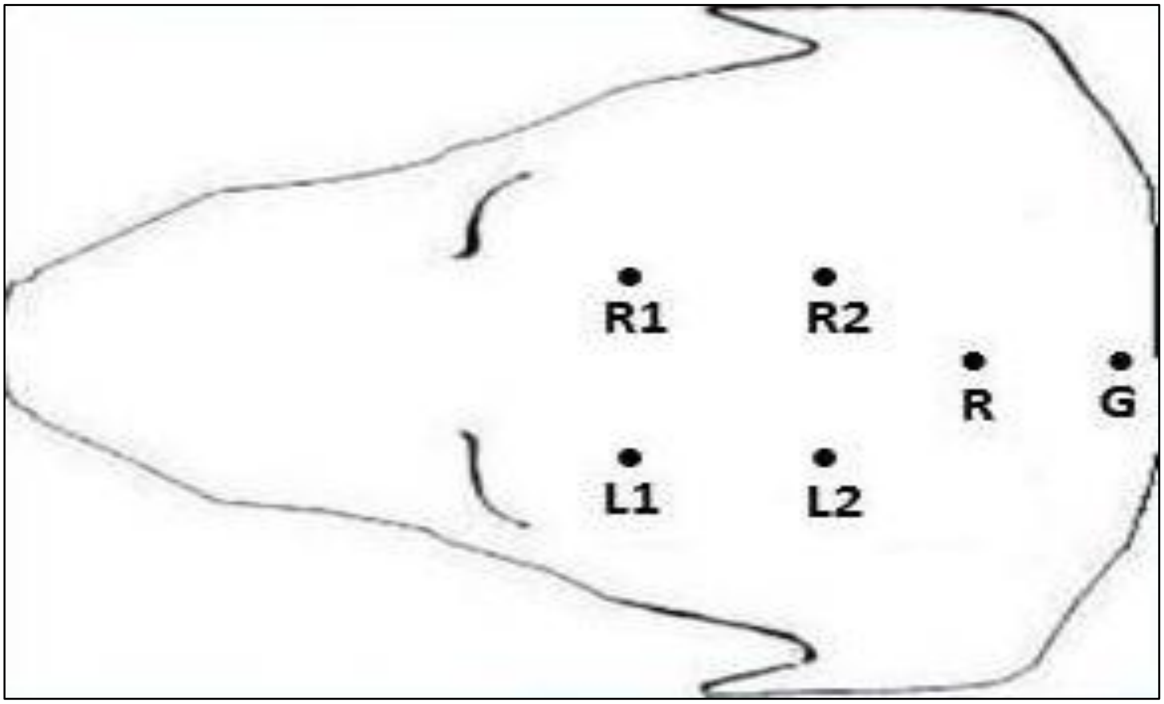

Figure 3. Left (L) and right (R) anatomical montage of EEG electrodes according to a L1, L2, R1, and R2 leads including reference (R) and ground (G) terminal leads applied to the piglets cranium.

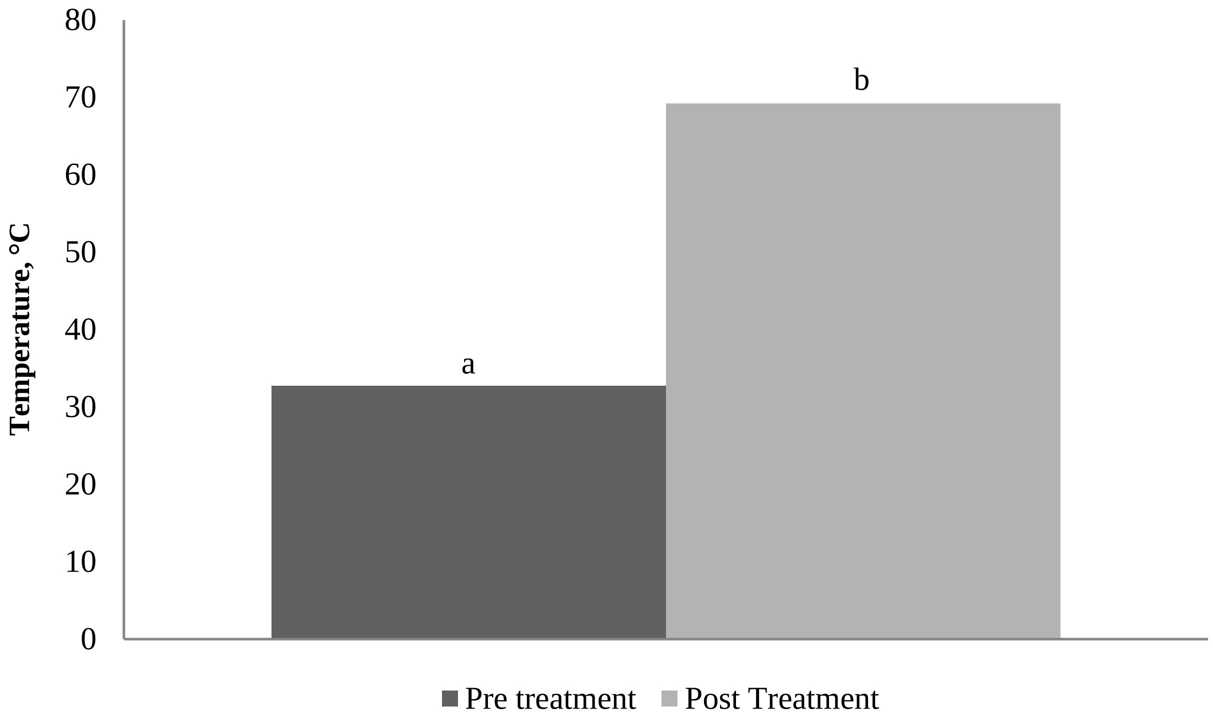

Figure 4. Effect of EM energy exposure on average head surface temperature of piglets.
$^{a, b}$Means with different scripts differ ($P < 0.01$).

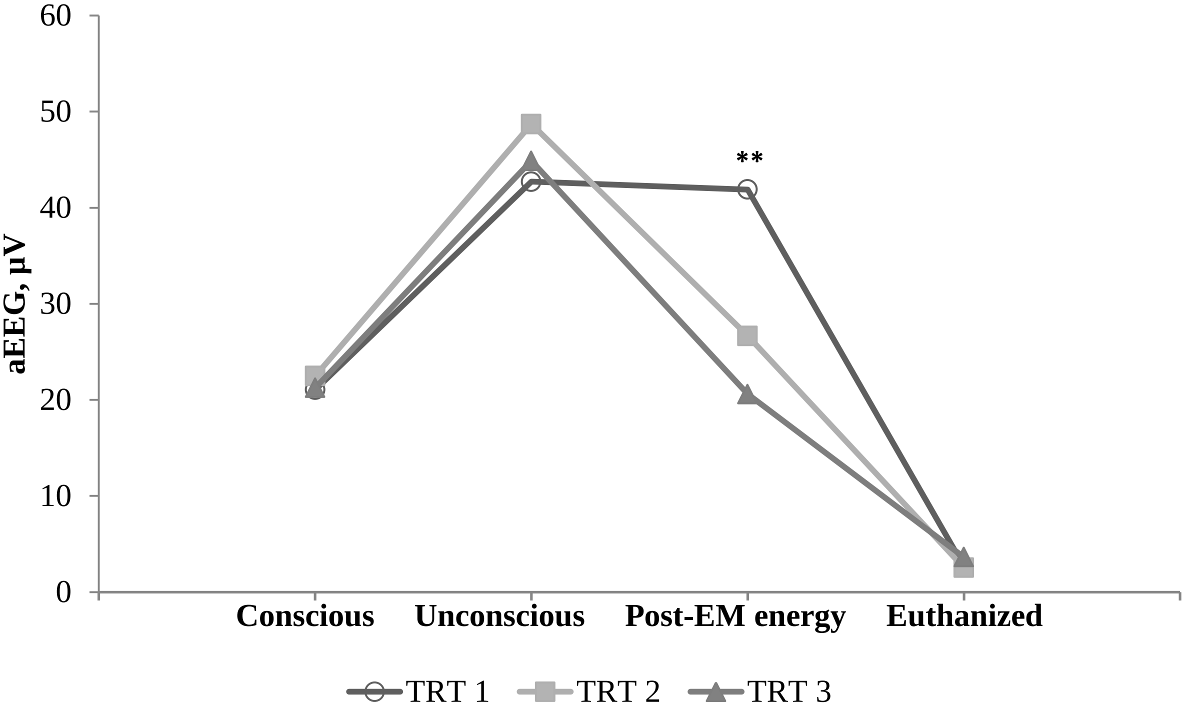

Figure 5. Effects of state of consciousness diagnosis of amplitude electroencephalogram (aEEG) across cranial application of electromagnetic (EM) energy administered for 3 (TRT 1), 6 (TRT 2) and 9 (TRT 3) seconds. TRT*StateCons = P < 0.01; SEM = 3.496.

**Indicates differences (*P* < 0.01) among treatments on specific state of consciousness.

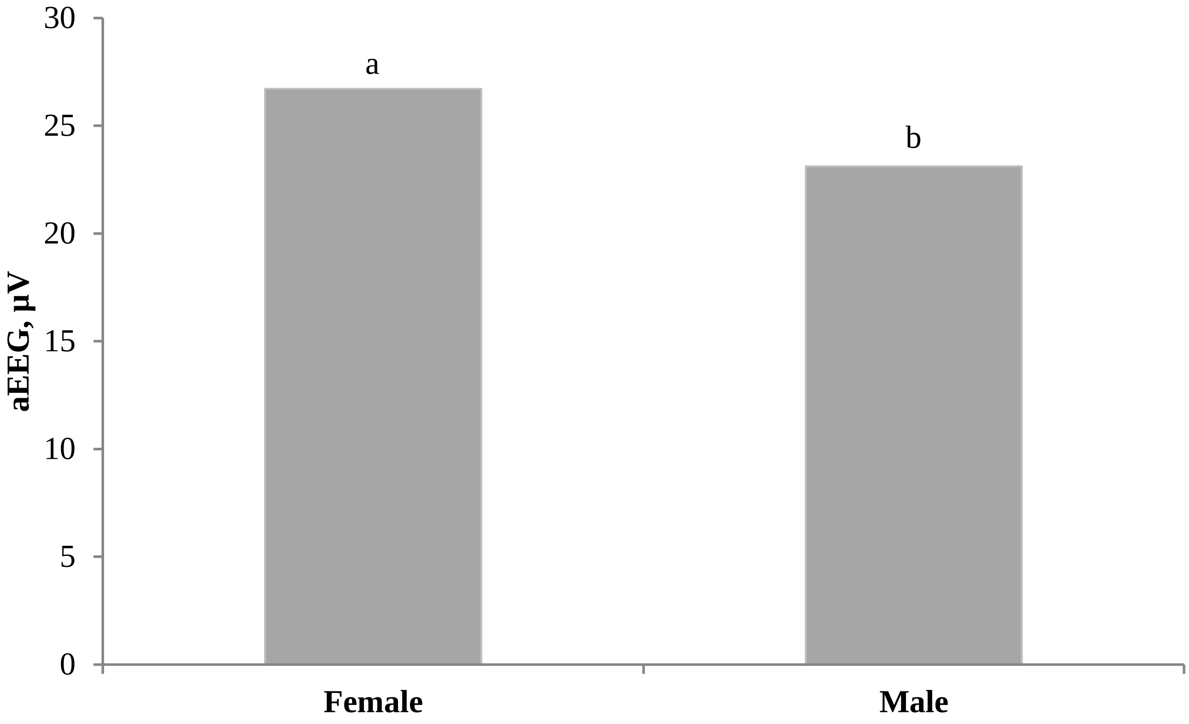

Figure 6. Male and female amplitude electroencephalogram (aEEG) differences evaluated as an average across all points of consciousness obtained prior to anesthesia, immediately after reaching a surgical plane of anesthesia (pupils fixed and dilated and no response to nose prick), and immediately after injection of chemical euthanasia (Euthosol®).

[a,b]Means with different scripts differ ($P < 0.03$); SEM = 1.076.

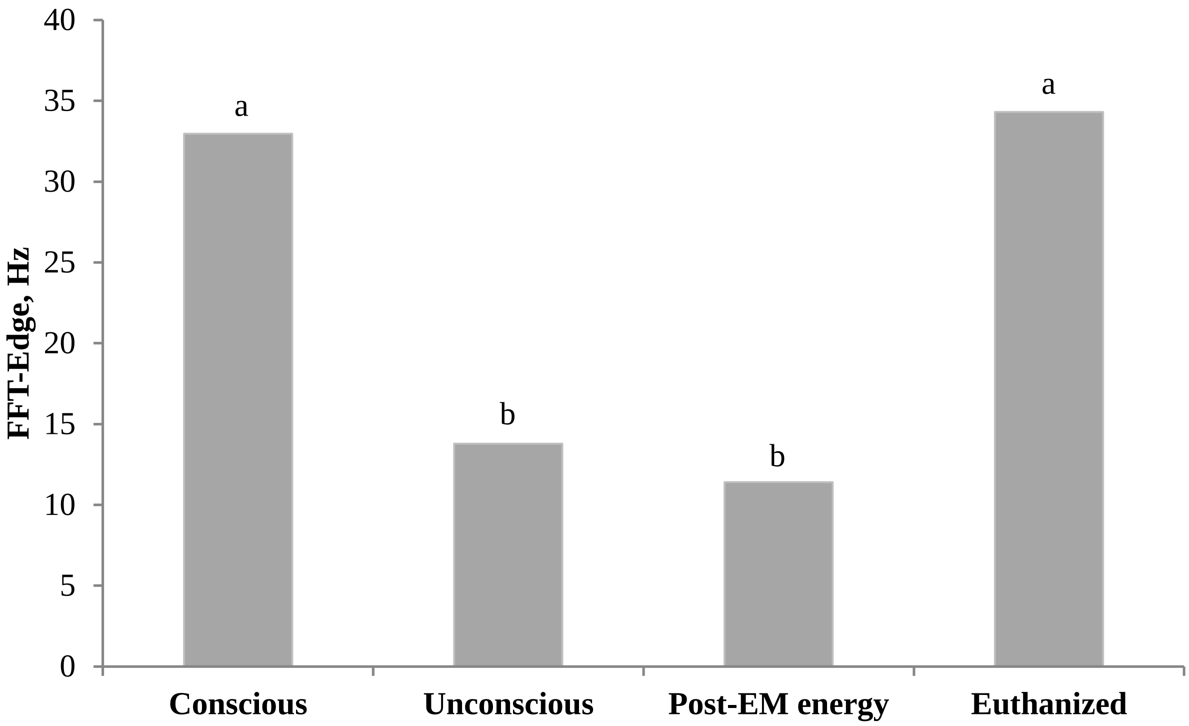

Figure 7. Effects of state of consciousness diagnosis of signal frequency electroencephalogram (FFT-Edge) of piglets obtained prior to anesthesia (Conscious), immediately after reaching a surgical plane of anesthesia (Unconscious; pupils fixed and dilated and no response to nose prick), immediately after cranial administration of electromagnetic (EM) energy (Post-EM energy), and immediately after being chemically euthanized (Euthosol®). ^{a,b}Means with different scripts differ ($P < 0.01$); SEM = 0.867.

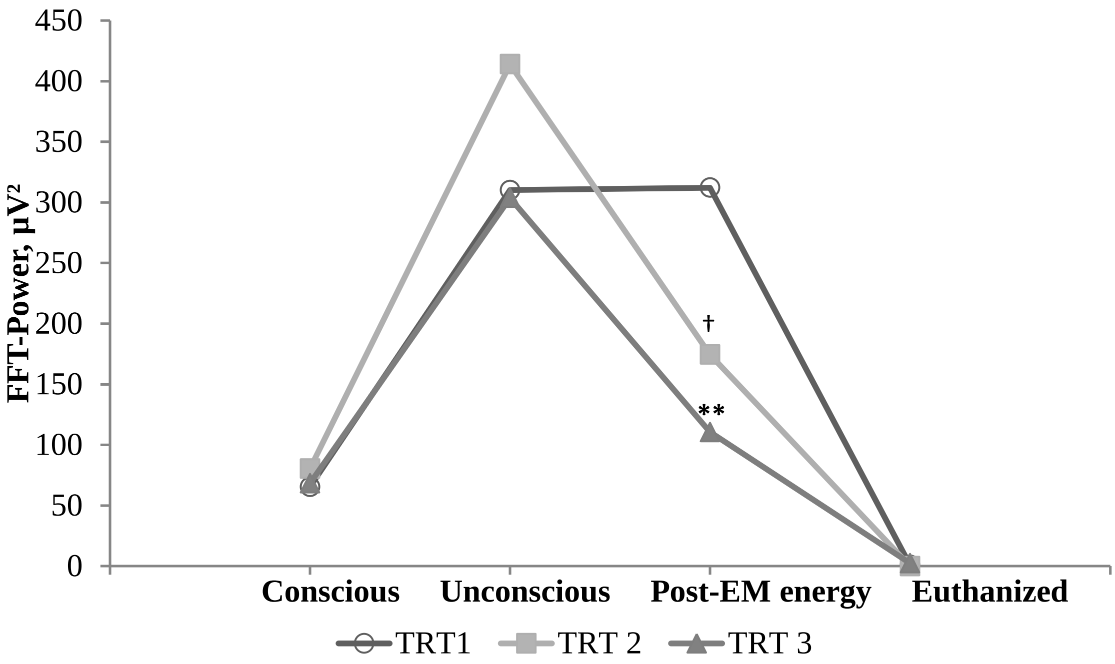

Figure 8. Effects of state of consciousness diagnosis of fast Fourier transform (FFT) power electroencephalogram across cranial application of electromagnetic (EM) energy administered for 3 (TRT 1), 6 (TRT 2) and 9 (TRT 3) seconds.

TRT*StateCons = $P < 0.01$; SEM = 3.496.

**Indicates differences between TRT 1 vs. TRT 3 at a specific state of consciousness ($P < 0.01$).

†Indicates differences between TRT 1 vs. TRT 2 at a specific state of consciousness ($P = 0.08$).

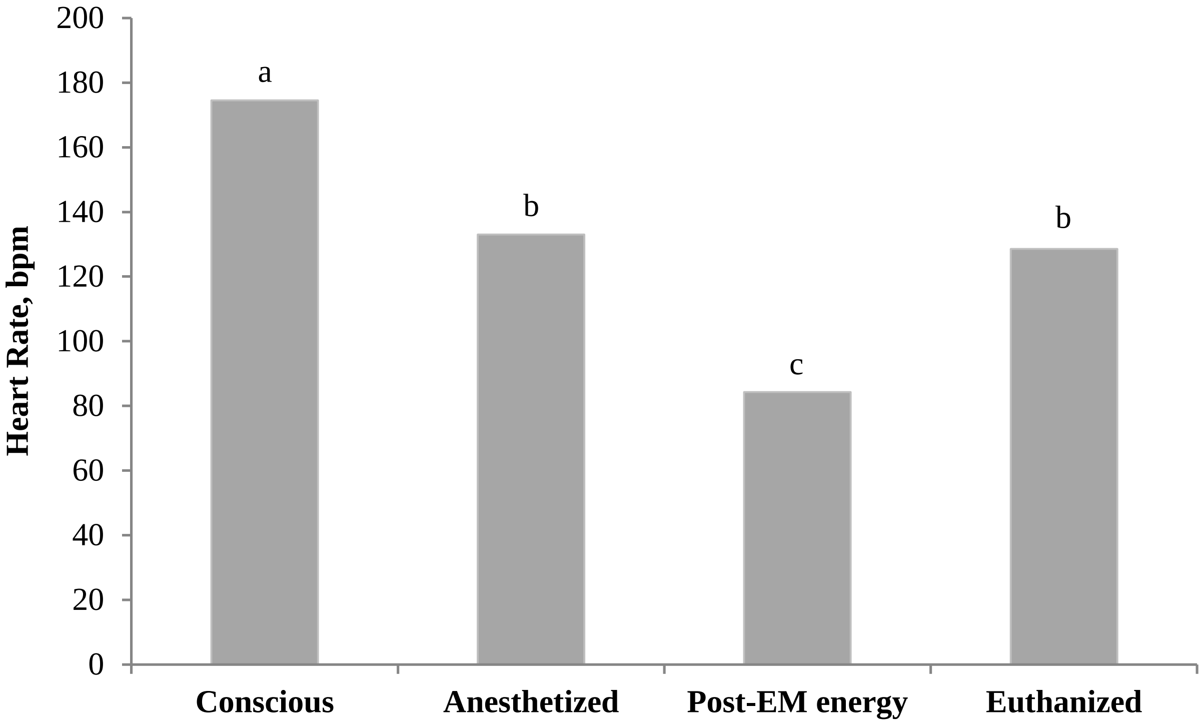

Figure 9. Effects of state of consciousness on heart rate of piglets obtained prior to anesthesia (Conscious), immediately after reaching a surgical plane of anesthesia (Unconscious; pupils fixed and dilated and no response to nose prick), immediately after cranial administration of electromagnetic (EM) energy (Post-EM energy), and immediately after being chemically euthanized (Euthosol®).

[a,b,c]Means with different scripts differ ($P < 0.01$); SEM = 13.440.

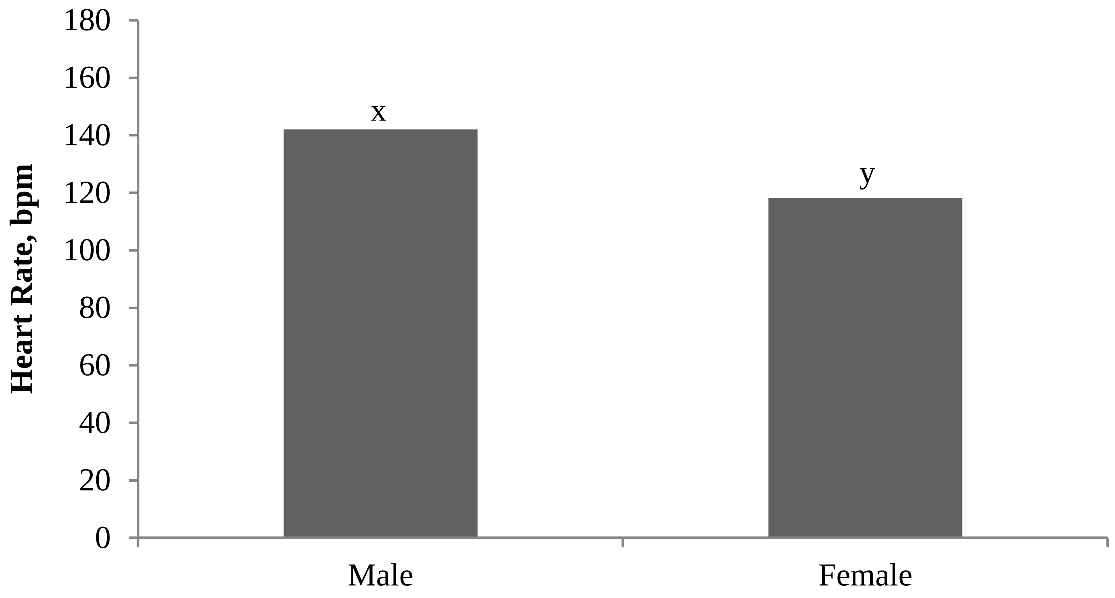

Figure 10. Male and female heart rate differences evaluated as beats per minute (bpm) averaged across all points of consciousness obtained prior to anesthesia, immediately after reaching a surgical plane of anesthesia (pupils fixed and dilated and no response to nose prick), and immediately after injection of chemical euthanasia (Euthosol®).

^{x,y}Means with different scripts differ ($P = 0.08$); SEM = 9.787.

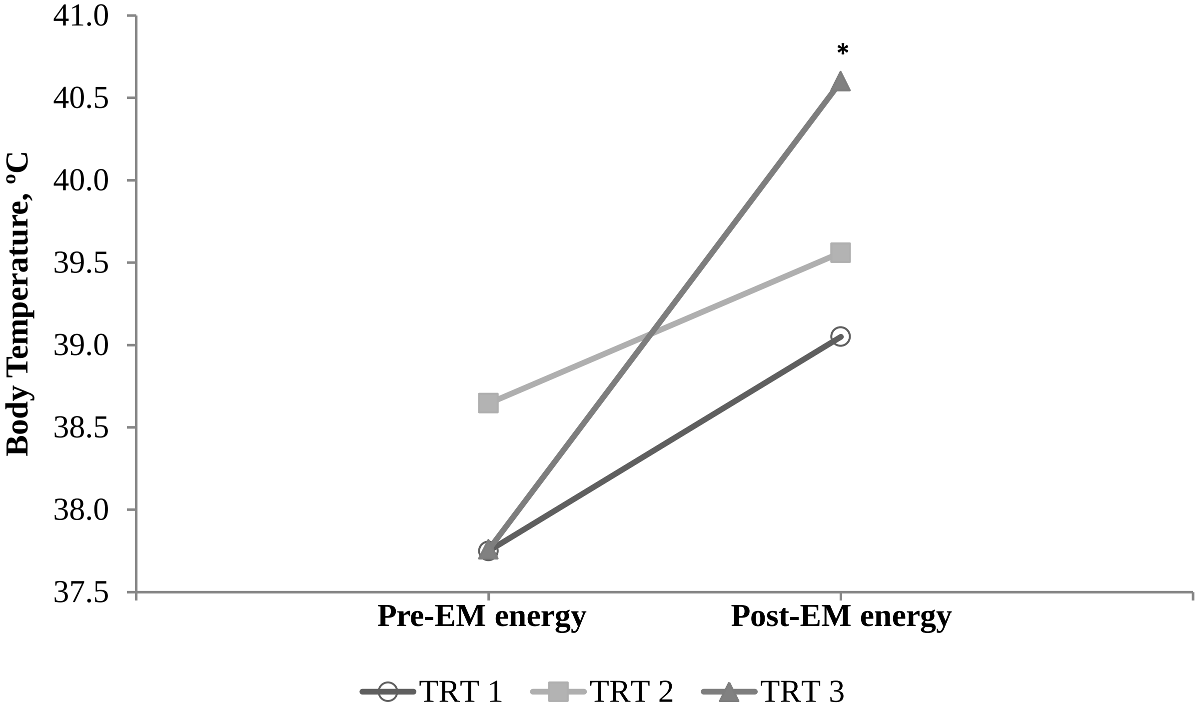

Figure 11. Effects of state of body temperature across cranial application of electromagnetic (EM) energy administered for 3 (TRT 1), 6 (TRT 2) and 9 (TRT 3) seconds.
*Means with different scripts differ ($P < 0.05$); SEM = 0.412.

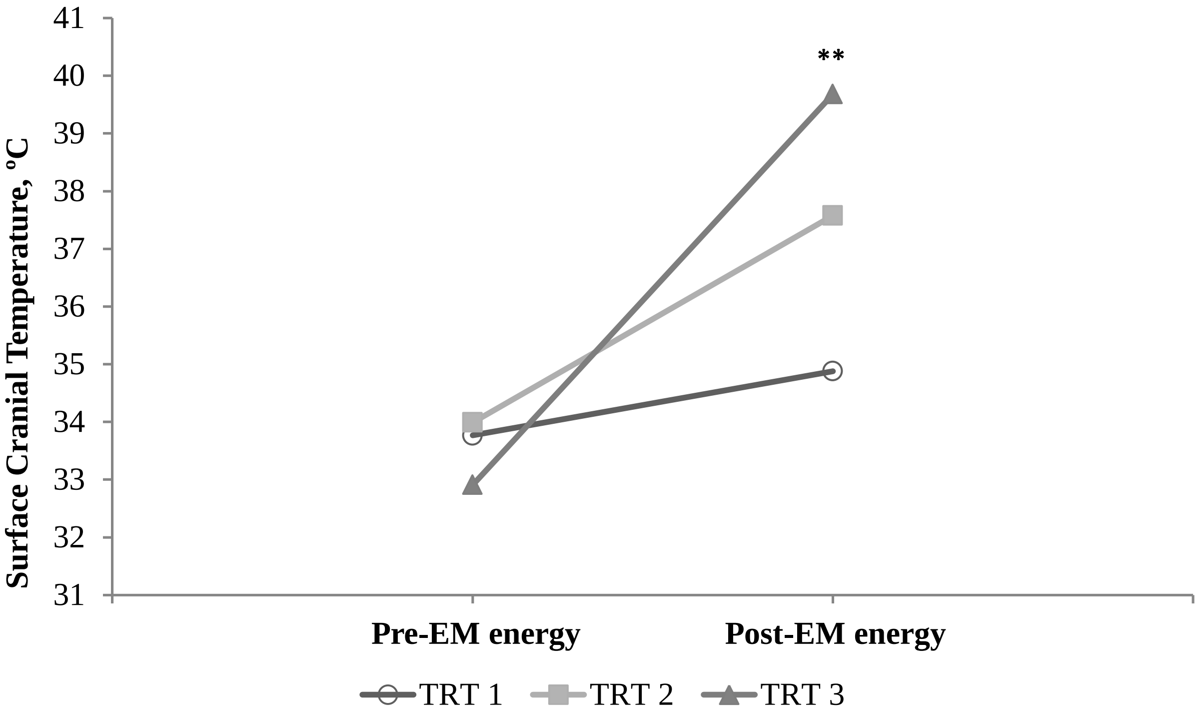

Figure12. Effects of state of surface cranial temperature across cranial application of electromagnetic (EM) energy administered for 3 (TRT 1), 6 (TRT 2) and 9 (TRT 3) seconds.
**Means with different scripts differ ($P < 0.01$); SEM = 0.798.