EVALUATION OF A NOVEL MEANS TO EUTHANIZE PIGLETS

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MASTER OF SCIENCE

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ABSTRACT

Research was conducted to evaluate a novel means to euthanize piglets utilizing electromagnetic energy. Experiment 1 assessed if electromagnetic energy could result in a euthanized piglet and Experiment 2 evaluated states of consciousness of piglets. During Experiment 1, six piglets were exposed to 40 seconds of electromagnetic energy (EME). Respiration rate was reduced to zero in each piglet immediately following EME and unassisted death occurred after heart rate ceased within 4.8 minutes after EME in five of the six piglets. Treatments during Experiment 2 included EME for 3, 6, or 9 seconds. State of consciousness and treatment interaction affected ($P < 0.01$) electroencephalogram amplitude and power. State of consciousness affected ($P < 0.01$) electroencephalogram frequency and heart rate. Treatment and EME interaction also affected internal body and head surface temperatures ($P < 0.01$). Piglets exposed to 9 seconds EME had higher ($P < 0.01$) body temperature compared with other treatments.

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CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE

Introduction

The swine industry is a progressive field which continuously strives to improve production practices including animal welfare. The term "welfare" refers to the state of an animal in relation to its environment (Broom, 1991). Animal research standards specify that any procedure that would cause pain or distress in humans should be assumed to do so in non-human animals as well (Organization for Economic Cooperation and Development 2000, U.S. Department of Agriculture 1997, and Public Health Service 1986). Public interest in animal welfare is growing and has inspired numerous discussions and debates about U.S. farm animal welfare policy (Croney and Millman, 2007). While industry stakeholders including producers, processors, and researchers continue efforts to maintain and improve welfare, it is still without argument that all producers involved in pork production are faced with the necessary task of euthanizing animals in order to alleviate animal suffering due to illness and/or injury. More specifically, piglet euthanasia is an inevitable part of raising swine within the pork industry. As producers are faced with the task of euthanizing animals on the farm, they are also faced with challenges that can be associated with the various types of euthanasia that currently take place within the swine industry.

Euthanasia can be defined as "humane death occurring without pain or distress" (Andrews et al., 1993). A humane death occurs without pain or distress and the welfare of the individual animal should be greatly considered (Gerritzen et al., 2004). The types of stress affecting livestock can be divided into two distinct categories; psychological and physical. Examples of psychological and physical stresses include restraint, handling, or novelty and hunger, thirst, fatigue, or energy respectively (Grandin, 1997). Moreover, when evaluating and choosing an appropriate method of euthanasia many factors should be considered including human safety, pig welfare, practicality/technical skill requirements, cost, aesthetics, and other limitations. With these factors and a multitude of animal welfare concerns constantly arising, it is difficult to define some methods of euthanasia as being consistently successful and noncontroversial. Thus, answering questions about the welfare of animals requires scientific definition and assessment (Swanson, 1995).

As the industry continues to combat specific welfare issues with regards to piglet euthanasia there will be a continued need for research and development of improved techniques.

Welfare and Euthanasia in the Swine Industry

As previously mentioned, animal welfare issues have become the forefront issue in food animal production and the swine industry has been a primary target of concern on multiple issues. With reference to piglets, there has been research published regarding the effects of processing procedures for animal husbandry and welfare; however, limited information and scientific studies can be found with regards to euthanizing piglets. Most research published on piglets relates to postnatal husbandry and the well-being of the piglet (Hansson et al., 2011; Marchant-Forde et al., 2009; and McGlone and Hellman, 1988).

There has been intense discussion of the current approved means of euthanizing piglets and there are different findings and beliefs based on analysis and methodology; however, little can be discovered in literature with relevance to animal pain. Moreover, as results among studies indicate that there are problems that arise while carrying out currently approved methods of euthanizing piglets.

Methods for Euthanasia of Piglets

The National Pork Board (NPB) and American Association of Swine Veterinarians (AASV) have compiled a list of approved methods of euthanasia including carbon dioxide asphyxiation, gunshot, captive bolt, electrocution, anesthetic overdose, and blunt trauma (AASV, 2008). However, the current and approved methods of euthanasia pose some faults and concerns; therefore, NPB and AASV support additional research on methods of neonatal euthanasia. Current methods can be considered stressful to the animal and aesthetically objectionable to people administering or observing the euthanasia technique. Additionally, captive bolt, electrocution, and blunt force trauma require a certain degree of skill and experience to be administered effectively and humanely on the first application. Likewise, anesthetic overdose, gunshot, captive bolt, electrocution, and carbon dioxide asphyxiation (to a certain degree) pose a potential danger to the worker or farm employee.

Chevillon et al. (2004) assessed common piglet euthanasia techniques by utilizing ten to 15 animals per category and outlined their success and efficiency by assessing stress and pain via vocalization. Death was assessed by collapse of the animal, convulsions, limb reflex responses, lack of movement, and measurement of heart beat and respiration rate. Carbon dioxide proved to be successful and efficient as all piglets assessed presented cardiac arrest in less than six minutes; but, loss of consciousness was not immediate and vocalization was observed. Carbon dioxide euthanized piglets appeared to undergo distress and pain; however, the piglets did become motionless in less than one minute. On the contrary, Chevillon et al. (2004) also evaluated blunt trauma to the head with a 0.5kg instrument and concluded that when this method was properly performed, loss of consciousness was immediate and the animal became motionless in less than one minute and thirty seconds.

Blunt Trauma

A blow to the head can be a humane method of euthanasia for neonatal animals with thin craniums, such as young pigs (AVMA, 2007). Blunt trauma can be defined as a quick, firm blow to the top of the head over the brain. Blunt trauma is only effective for suckling piglets (<12 lb) because their skull bones are thin enough for the force to cause depression of the central nervous system and brain damage. It is essential that the blow is administered swiftly, firmly, and with absolute determination ensuring euthanasia and not just loss of consciousness (AASV, 2008).

When properly performed, blunt trauma results in a rapid loss of consciousness followed by death. During blunt trauma, the pig will usually show tonic movements where the body becomes extremely tense followed by gradual relaxation. As relaxation sets in, involuntary kicking or paddling movements occur for a minute or two prior to death (AVMA, 2007).

Whiting et al. (2011) examined various methods of rapid mass euthanasia of segregated early weaned piglets including manual and controlled blunt trauma. Whiting's group reported that manual blunt trauma proved to be difficult to apply to practice. As well, immediately after the manual blow, an unconscious piglet is difficult or impossible to differentiate from a conscious brain-injured piglet struggling in response to pain. On the contrary, the application of controlled blunt trauma resulted in a high level of confidence in operators administering method as a piglet with a dramatic physical indentation in the dome of the skull was unconscious and could be placed on the floor.

Carbon Dioxide

Carbon dioxide (CO_2) asphyxiation is a common method of euthanizing piglets (<70 lb) on the farm (AASV, 2008). At concentrations above 60%, CO_2 acts as an anesthetic agent and causes rapid loss of consciousness (Close et al., 1996 and Green et al., 1981). Carbon dioxide euthanasia occurs via administration of the inhalant gas in a sealed container with the purpose of inducing unconsciousness and death (Britt, 1986). Euthanasia by CO_2 inhalation is relatively inexpensive; but, requires special equipment to work properly. In addition, euthanasia of swine via CO_2 asphyxiation is considered a safe method of euthanasia for farm personnel to perform (AASV, 2008).

To date, literature primarily focuses on effects of CO_2 asphyxiation in market hogs as a means of stunning hogs prior to exsanguination. Raj (1999) examined the welfare implications of mixtures of gases (argon and CO_2) and the time required to stun and kill pigs. The paper states that CO_2 resulted in shorter respiratory arrest time. Additionally, all pigs exposed to the gas mixture died following seven minutes of exposure.

In addition, CO_2 is the most commonly used agent for euthanasia of laboratory rodents, largely because of its ease of use, relative safety, and low cost (AASV, 2008).

Anesthetic Overdose

The use of injectable euthanasia agents is the most rapid and reliable method of performing euthanasia (AVMA, 2007). Veterinarian administered euthanasia solutions (i.e. barbiturates) are utilized in a way to depress the central nervous system resulting in deep anesthesia progressing to respiratory and cardiac arrest (death). Anesthetic overdose is done by injecting the solution intravenously into the pig (no weight limitations). Solutions utilized for anesthetic overdose are required to be under the supervision of an individual who is registered with the U.S. Drug Enforcement Administration (AASV, 2008).

Special attention must be utilized when utilizing anesthetic overdose as a means of euthanizing on the farm in order to prevent exposure of the chemical residues in the carcass to other animals on the farm.

Quantifying an Effective Euthanasia

Traditionally, from an animal welfare point of view, observation of a successful euthanasia has been observed through physical measurements. Regardless of method used, it is important to be able to recognize ineffective euthanasia if it occurs. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest (AVMA, 2007). 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 (Grandin, 2001a).

To date, with regards to euthanizing and stunning meat animals, the determination of suffering and sensibility has been based on subjective measurements such as head movement, leg kicking, and gasping (AVMA, 2007). As a result of the complicating task of analyzing and evaluating subjective measurements in the scientific field, it is important to utilize non-subjective measurements as a means of analyzing a euthanasia project with regards to animal pain and suffering. Factual knowledge is required to support ethical decisions and is described below.

Insensibility

Insensibility should be checked 30 seconds post method of administration and should be monitored and maintained until death occurs. The presences of rhythmic breathing, constricted pupils, righting reflex, vocalization, palpebral reflex, or response to painful stimuli are signs of sensibility and an infective stun or euthanasia (AVMA, 2007).

Gerritzen et al. (2004) observed on-farm euthanasia success by measuring behaviors including head shaking, side-to-side head movement, gasping, loss of posture, and convulsions. Although widely used, this methodology is subjective to individuals watching and recording the measurements. Observation of behavior and reflexes in relation to stunning experiments is complicated by the fact that they do not give a definite answer about unconsciousness and level of pain (Lambooij et al., 1996).

A special report by Grandin (2001b), it is stated that "return to sensibility is done so in the order of eye reflexes, response to a needle prick on the nose, and return to righting reflex". Grandin (2001b) also stated that untrained personnel have problems misinterpreting eye reflexes and eye reflexes are of the utmost importance, because an animal with a responsive eye has started the process of return to sensibility.

An alternative approach for determining brain dysfunction and essentially determining insensibility is recording brain impedance (Van Harreveld, 1972). Changes in brain impedance reflect changes in the extracellular volume of the brain due to metabolic failure of the cells (Savenije et al., 2002). Savenije et al. (2002) utilized brain impedance recordings to measure brain damage in electrically stunned chickens by attaching a pair of silver electrodes in the striatum area of the brain and connecting them to an impedance-recording device.

Death

A piglet should be confirmed dead before it is moved for disposal. Vital signs (respiration, heartbeat, muscle movement, response to painful stimuli, vocalization, and corneal reflex) should be checked and cease three minutes following the application of the euthanasia method has been applied (AASV, 2008). The most important aspects in recognition of death include cessation of heart beat and respiration and absence of reflexes (Close et al., 1996). If animals show any signs of sensibility or life it is recommended that a backup method is utilized (AASV, 2008 and Close et al., 1996).

Electroencephalogram

Based on scientific and anecdotal evidence, it is important to identify a method that can determine animal pain, suffering, insensibility, and unconsciousness. "To date, there appears to be no distinct division between conscious and unconscious states; therefore, the criteria for properly euthanized piglets should be quite conservative and evaluation of new electrical specifications must be performed in a laboratory in which a measurement of brain activity may be monitored" (Grandin, 2001b). In **The Handbook of Veterinary Pain Management**, Gaynor and Muir (200) state "no 'gold standard' exists to assess pain in animals or to compare one type of scale measurement instrument to another". Additionally, all pain scales have a subjective measurement and are vulnerable to observer error and bias (Gaynor and Muir, 2009).

Drawbacks of determining insensibility by means of subjective observations previously mentioned can be avoided by measuring the electrical activity of the brain by an electroencephalogram (EEG; Lambooij, 1981). Electroencephalogram is the graphic recording of the rhythmic bioelectric activity arising predominately from the cerebral cortex (Lukatch et al., 1997). A profound suppression of bioelectric activity (90% reduction) of pre-stun levels constitutes a state of unconsciousness and insensibility (Lukatch et al., 1997) while death reveals no activity. In postnatal animals, absence of EEG activity is regarded as evidence of lack of consciousness and the insensitivity of an animal to pain (Croft, 1952).

Parameters of EEG include frequency and amplitude. In terms of frequency, brain waves can be categorized into four basic groups including: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (>13 Hz). These wave lengths may also vary across clinics; however, those listed are commonly accepted (Kaiser, 2006). Alpha activity is induced by closing the eyes and by relaxation and abolished by eye opening or any activity such as thinking. During normal wakefulness, beta waves are dominant (Teplan, 2002). Generally speaking, average amplitude reading for an adult human ranges from 10µV to 100 µV (Woo Lee and Khoshbin, 2008).

With respect to human research, EEG technology has been utilized extensively as a means of assessing and monitoring the depth of anesthesia (Zhang et al., 2001; Heier and Steen, 1996; and Stanski, 1994). Newhook and Blackmore (1982c) utilized EEG to determine insensibility in sheep and lambs at the time of slaughter. The study interpreted EEG based on amplitude and pattern of fast wave signals.

Newhook and Blackmore utilized EEG in a multiple part study to determine the onset of permanent insensibility in sheep and calves during slaughter (Newhook and Blackmore, 1982a; Newhook and Blackmore, 1982b; and Newhook and Blackmore, 1982c). Part 1 of Newhook and Blackmore's study (1982a) described EEG recordings in the bipolar mode with two of the electrodes active and a third electrode acting as a reference electrode. Part 1 further describes recordings based on amplitude under different conditions including excited, alert, relaxed, resting, somnolent, during the induction of anesthesia, and during the recovery of anesthesia. Newhook and Blackmore (1981a) utilized their EEG recordings to conclude that insensibility occurred in sheep 2 - 65 seconds and death occurred 10 - 375 seconds post severance of major blood vessels. In part 2 of their study, Newhook and Blackmore (1982b) utilized the same methodology as previously mentioned and concluded that continuous sensibility occurred for 65 - 85 seconds and calves had brief periods of sensibility 120 - 323 seconds after slaughter.

In terms of market hog EEG research, Martoft et al. (2001) utilized EEG technology to determine the effects of CO_2 anesthesia on the central nervous system in swine. Additionally, a

German group measured the effects of CO_2 stunning on EEG readings in order to determine the sufficiency of a stun (Hartung et al., 2002).

Additionally, Rose et al. (1972) studied EEG patterns in awake and anesthetized pigs. EEG was recorded on 62 awake pigs ranging from 1 hour to 12 months of age. Further, EEG was recorded on 51 anesthetized pigs between 10 and 14 weeks of age. When comparing the different ages of pigs, the group found that formation of fast rhythms and the lessened amount of the slower frequencies were a function of the maturation of the pig. The group did not compare awake and anesthetized EEG on the same pigs.

With regards to anesthetics, EEG can be utilized to measure the depth of anesthesia. Voss and Sleigh (2007) states that anesthetic agents are accompanied by a shift from low-amplitude, high- frequency EEG to high-amplitude, low-frequency activity. Additionally, Bo et al. (2003) reported similar findings (slow waves of high amplitude) in a study utilizing quantitative EEG (qEEG) analysis to monitor general anesthesia in rats.

Quantifying EEG

Advances in computational techniques have led to the development of qEEG, which allows a more objective evaluation and analysis of cerebral electrical activity (Otero et al., 2011). Quantitative methods for analyzing EEG provide new ways to view data that are different from the traditional visual analysis. The field of qEEG could not emerge until machines could assist in the analysis (Brazier, 1961). Quantitative EEG is a general term for analysis of the EEG, generally with a mathematical formula. Computers allow for the digitizing of signals recorded from the scalp and identification of specific electrical wave patterns within each signal. Additionally, computers display the EEG patterns on a computer screen and store them within seconds and then transform the data in ways that highlight certain features that may be difficult to assess by only visual inspection (Kaiser, 2006).

qEEG analysis generate variables that include amplitude, frequency, and power. These variables can be used to help establish the presence and severity of cerebral activity (John et al., 1988).

Lewis et al. (2011) utilized computer-aided analysis to evaluate EEG in young cats. The group found that amplitude and power decreased as cats aged to 24 weeks and that age had no effect on frequency; thus, age was an important consideration when interpreting EEG data.

Use of Electromagnetic Energy as a Method of Euthanasia

Microwave irradiation is utilized primarily by neurobiologists to fix brain metabolites; but, still maintaining the anatomic integrity of the brain. Microwave instruments 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 microwave energy to the head of the animal essentially targeting the brain (AVMA, 2007).

Guy and Chou (1982) utilized a cylindrical restraining device in an electromagnetic (EM) energy rat model to test microwave induced unconsciousness on 71 anesthetized rats. The apparatus utilized consisted of a circular waveguide whereby the animal's body was positioned in a cone which restrained the entire body and placed the head in a region of high EM field strength. With regards to EEG and electrocardiograms (EKG), Chou and Guy (1982) measured these two parameters utilizing implanted carbon surface electrodes before and after exposure to EM energy. In anaesthetized rats, there was little or no frequency changes after the exposure; however, in anesthetized rats, the EEG amplitude increased several fold and recovered within two minutes after exposure. Further, EKG results were similar to EEG results as anaesthetized animals experienced decreased heart rate and anesthetized animals displayed increased heart rate.

A group in Germany utilized microwave irradiation as a more humane way of stunning chickens (Zeller et al., 1989). The project results indicated that chickens can be killed in less than one second by exposing their heads to microwave irradiation; but, positioning of the head must be precise. Further, the application methodology included hanging chickens up headlong and this could be categorized as a considerable stress. As a result, it could be suggested to prefer anesthesia before hanging chickens along the conveyor belt.

Ikarashi et al. (1984) studied the use of the microwave magnetic field for the rapid inactivation of brain enzymes by exposing rats and mice to energy from a microwave device with a power of 10 kW at 2,450 MHz. The group found that 330 milliseconds following microwave energy resulted in a brain temperature of 79°C in mice. Similarly, the group reported a brain temperature of 94°C in rats following 800 milliseconds of microwave energy.

Although unconsciousness may be rapid and successful, the power required to rapidly halt brain enzyme activity depends on the efficiency of the unit, the ability to tune the resonant cavity, and the size of the animals head (AVMA, 2007 and Stavinoah et al., 1978).

EM energy presents a novel approach to the task of euthanizing piglets and research should be conducted in order to discover its further implications.

CHAPTER 2. EVALUATION OF A NOVEL MEANS TO EUTHANIZE PIGLETS

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. Each of the currently approved methods for euthanizing piglets has raised 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).

It is thought that euthanasia by means of EM energy has the potential to eliminate negative concerns related to euthanasia of piglets. The seven considerations as defined by National Pork Board and American Association of Swine Veterinarians are outlined (Table 1) along with a description of how EM euthanasia addresses each of the considerations (AASV, 2008). There is a potential for EM euthanasia to address each of the seven concerns outlined by the NPB and AASV.

Table 1. Euthanasia Considerations

NPB/AASV Considerations	EM Euthanasia Address this Concerns
Human safety: The method must not place producers or their employees at unnecessary risk.	EM energy is fully contained and poses no risk to the administrator and piglet restraint device will be utilized to immobilize piglets prior and during application of euthanasia eliminating human contact.
Pig welfare: The method should minimize any pain or distress on the animal.	EM energy will be administered with enough power to increase brain temperature to a level resulting in complete necrosis of brain cells from the frontal lobe to the brain stem in 2 to 4 seconds. The frontal cortex is the portion of the brain that recognizes pain; the brain stem is responsible for vital life functions (including respiration). The ability to detect pain is the first region of the brain will be rendered inoperable and will occur nearly instantaneously. Complete euthanasia follows upon lysis of the brain stem cells and neurons.
Practicality/technical skill requirements: The method should be easily learned and repeatable with the same expected outcome.	A piglet restraining device must be developed to immobilize the piglet and to position the head consistently for application of the EM pulse for each application.
Cost: The method should be economical for the producer.	The initial cost of the equipment is a one-time expenditure and becomes the only expense eliminating the cost of CO_2 or other expendable materials utilized in other forms of euthanasia.
Aesthetics (degree of unpleasantness for the observer): The method should not be objectionable to the person administering the procedure.	Piglet is placed in containment unit and is removed euthanized, resulting in a bloodless form of euthanasia.
Limitations: Some methods are only suitable for certain sizes of pigs or certain locations.	This methodology could only be administered to post-farrowing or nursery piglets. Larger pigs would require more energy, much larger equipment, and a much more intensive means of restraint.

Recent research conducted at North Dakota State University has yielded promising results using 1.4 KW of EM energy administered in the microwave range focused on the frontal lobe of the piglet brain (unpublished data). Based on the results from this preliminary research, an increase in power level of EM energy will considerably reduce the application time, further elevate intra-cranial temperature, and result in complete brain necrosis from the frontal lobe to the brain stem.

The short/long term economic implications of negative public perception associated with current techniques used for on-farm euthanasia are difficult to quantify. As animal welfare issues continue to advance to the forefront, the swine industry must address these matters in a proactive way. Development of a novel means of piglet euthanasia that eliminates negative images of blunt trauma or group asphyxiation could be the scientific breakthrough that further protects the U.S. pork industry.

Moreover, the utilization of electroencephalogram (EEG) measurements poses a significant opportunity to establish baseline conscious, unconscious, and death state of animals. EEG technology presents an objective way to measure an animal's response to pain and sensibility and a better route to understanding animal welfare issues. More specifically, quantitative EEG (qEEG) can be utilized to compare various industry practices from a welfare point of view.

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 (Exp. 1). In addition, qEEG technology will be used to characterize the conscious, unconscious, post EM energy application, and death states in piglets and to aid in determining whether or not the piglets' death occurred without pain and sensibility after the administration of the EM energy (Exp. 2).

Materials and 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).

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 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. 1) 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; Braun™, 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 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.

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 thermal imaging camera (Fluke Ti 25; Fluke Corporation, Everett, WA, USA), initial body temperature was recorded using a thermoscan ear thermometer (Model IRT 4520; Braun™, 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. 2).

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 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 (μV^2), 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 theirs respectively 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 and 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 only slightly 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 was still beating after the predetermined threshold of 10 minutes.

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. 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 had an effect ($P < 0.01$) on average head surface temperature (Fig. 3). Average head surface temperature measurements were much higher following exposure to EM energy (32.7 vs. 69.2ºC, respectively).

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. These results indicate that nervous tissue denaturation occurred as a result of exposure to EM energy exposure. These results are further supported by literature that clearly states that temperatures above 41°C will cause proteolysis which would lead to nervous cells denaturation and loss of function (Gao et al., 2010; Fagain, 1997; and Branden and Tooze, 1998). Further, it can be concluded as nervous cells denature and lose function, the ability for an individual 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. 4). 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 were 2.66, 2.53, and 3.63 μV for treatments 1, 2, and 3, respectively. Amplitude values were much lower ($P < 0.01$) in treatments 3 compared with treatment 1 following EM energy application. This indicates three and six seconds exposure time does not affect EEG amplitude in a great amount and that time of exposure should confidently exceed six seconds.

Within each treatment, from the conscious to unconscious states piglets had an aEEG value 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, following cease of respiration aEEG was 90% lower compared with aEEG following exposure to EM energy.

With reference to treatment 1, 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, 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, there was also an increase in aEEG values from the conscious to unconscious state. aEEG values from the unconscious state to the state immediately following exposure to EM energy were higher. In contrast, values obtained from the conscious state and the state immediately following exposure to EM energy were similar. Amplitude EEG values obtained following cease of respiration were lower than values obtained in the other three states of consciousness.

Moreover, aEEG values 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 5. 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 greatly differ ($P < 0.01$) among the four states of consciousness (Fig. 6). 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. 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 7. 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. 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 interaction between treatment and state of consciousness and the main effects of sex will be mentioned below.

As can be seen in figure 8, 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 to be greater ($P = 0.08$) in male piglets compared with female piglets (142 vs. 118 bpm, respectively). This difference can be seen in figure 9.

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 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. 10). 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 head surface temperature (Fig. 11). Prior the application of EM energy, 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. 34.9ºC, 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; Fagain, 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 the 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 for improvements on-farm husbandry and euthanasia techniques with regards 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 utilized to 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 EM energy has on the sensibility of piglets. Based on the qEEG data from this study, it would be recommended that trials utilizing the same amount of EM energy to euthanize piglets in the future 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.

We conclude from experiment 2 that three seconds is not sufficient time to denature brain tissue to a point that eliminates perception of pain. In the future, if piglets are exposed to greater than six seconds of EM energy a better understanding of sensibility can be obtained. After piglets do reach a point of insensibility the duration of EM energy exposure could be carried to the point of complete brain denaturation and essentially death.

Figure 1. Effect of EM energy cycle on temperature change in the brain of deceased piglets

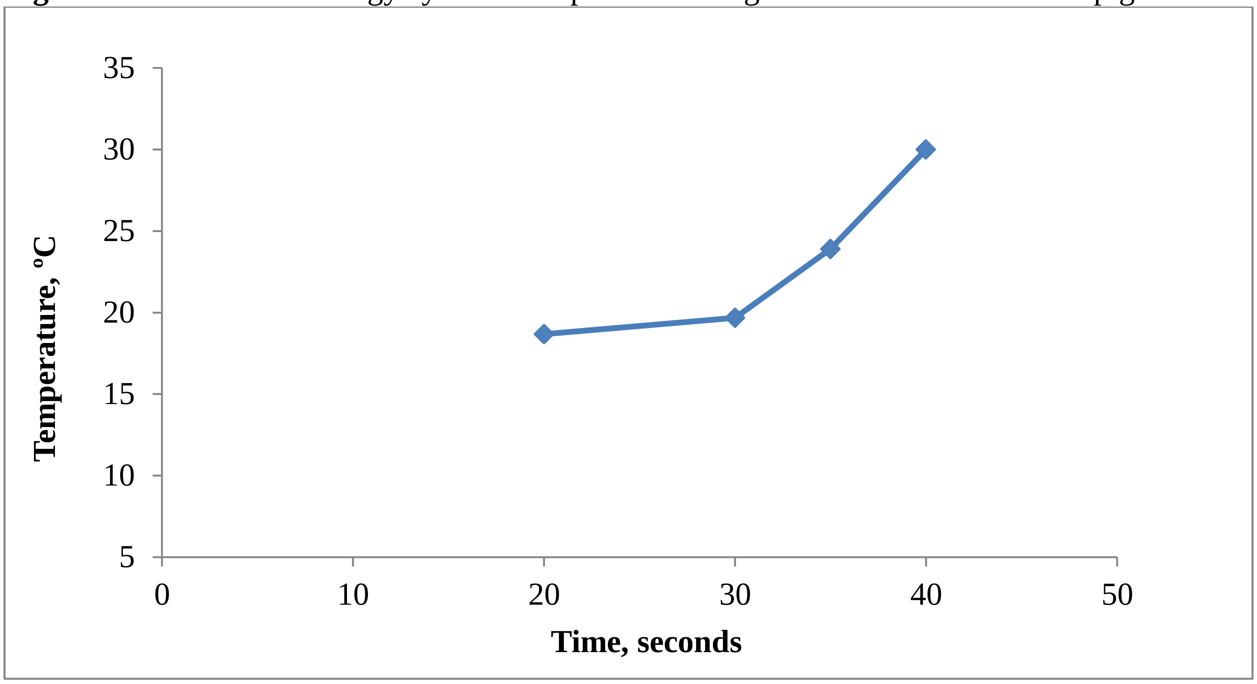


Figure 2. Anatomical location of EEG electrode leads on head of piglets

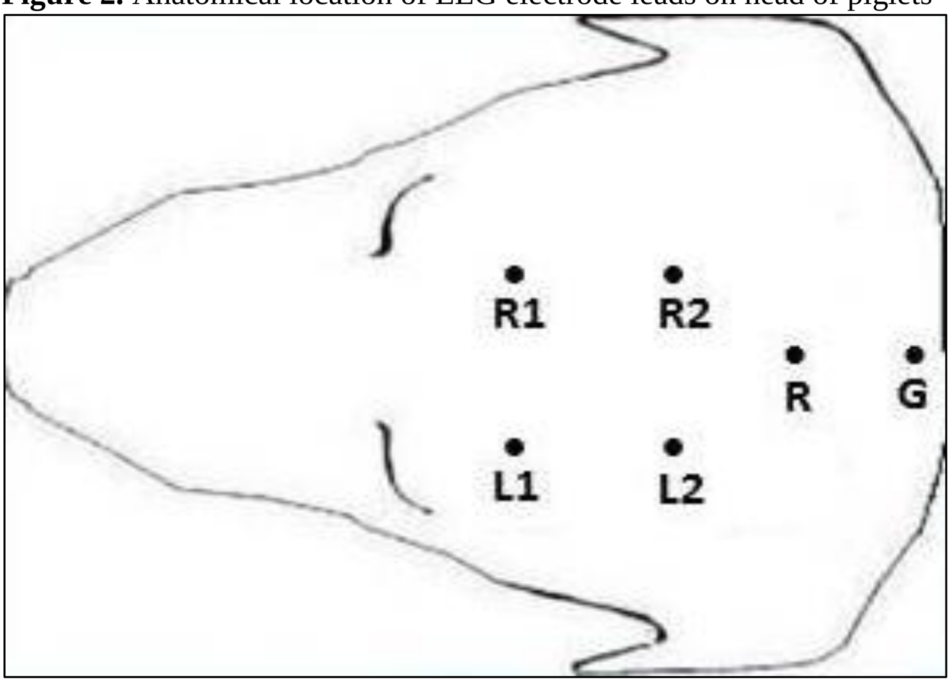


Figure 3. Effect of EM energy exposure on head surface temperature of piglets

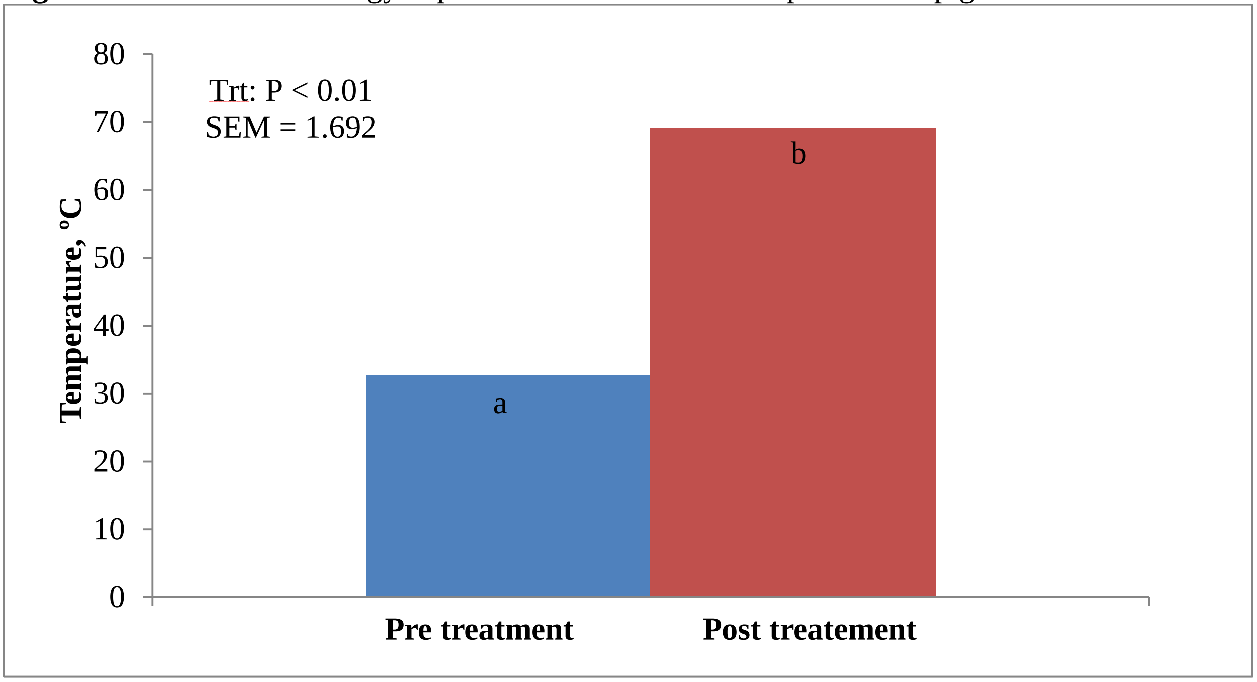
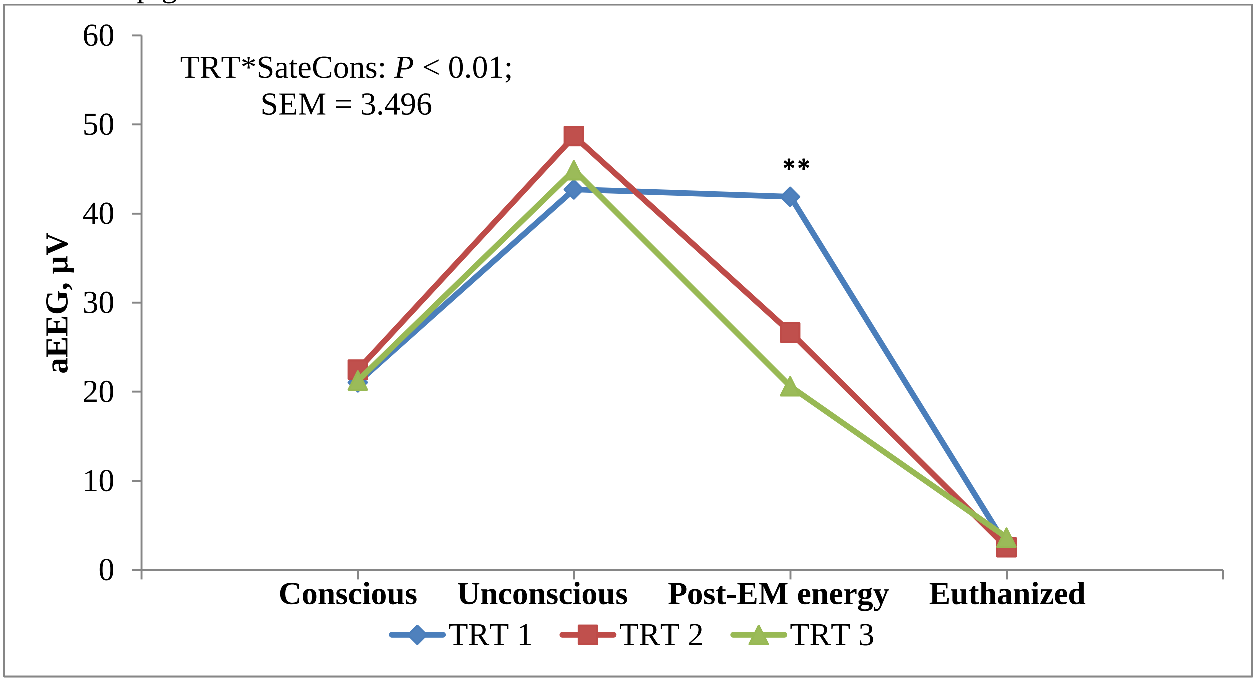


Figure 4. Effects of state of consciousness and treatment interaction on amplitude of EEG of piglets



** Indicates differences among treatments at a specific state of consciousness ($P < 0.01$).

Figure 5. Effects of sex on amplitude of EEG of piglets

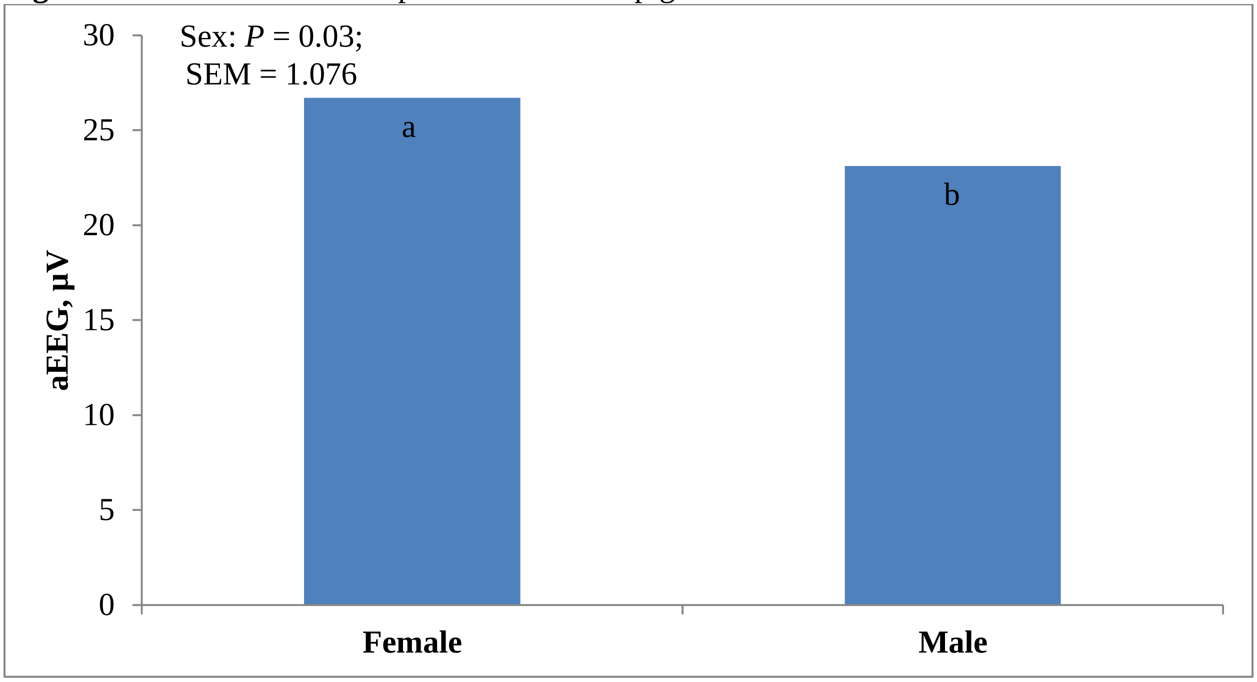


Figure 6. Effects of state of consciousness on frequency of EEG of piglets

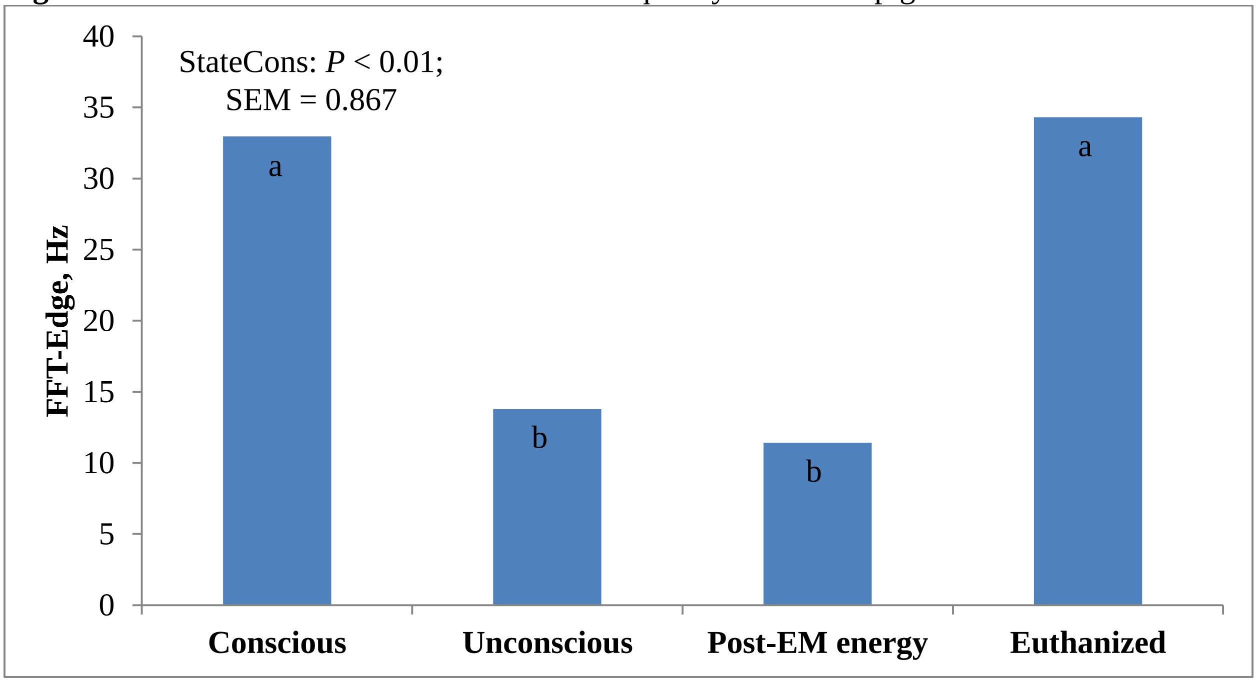
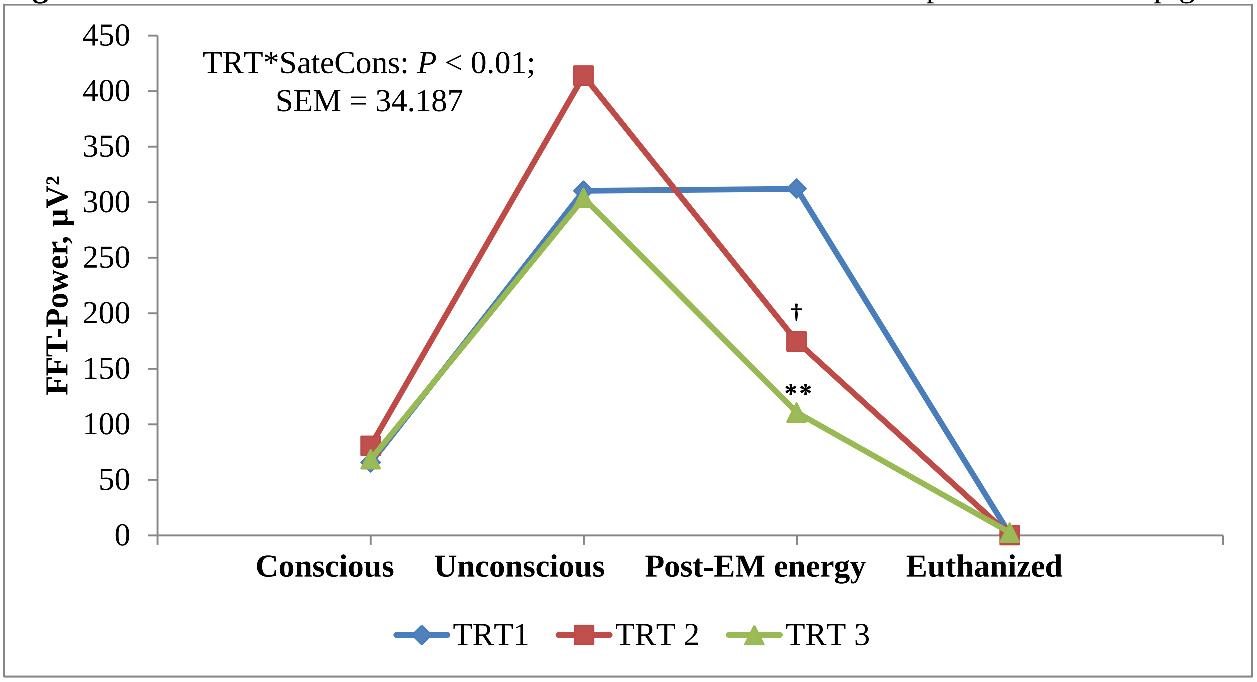


Figure 7. Effects of state of consciousness and treatment interaction on power of EEG of piglets



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

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

Figure 8. Effects of state of consciousness on heart rate of piglets

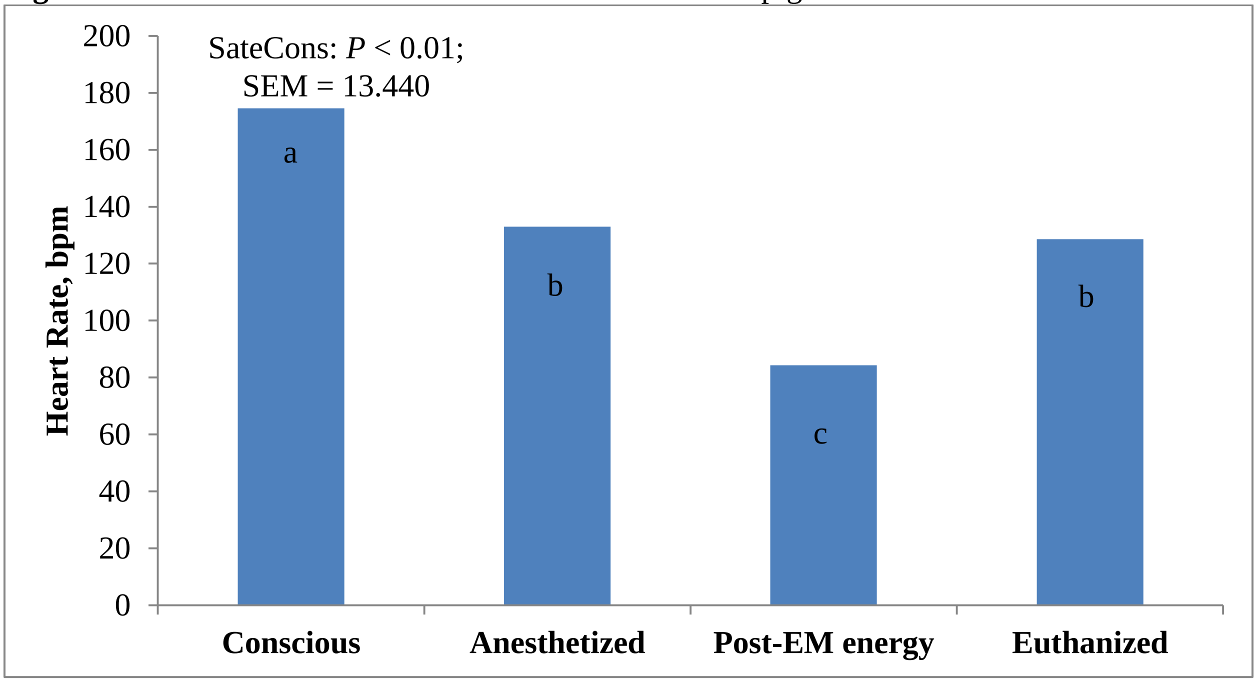


Figure 9. Effects of sex on heart rate of piglets

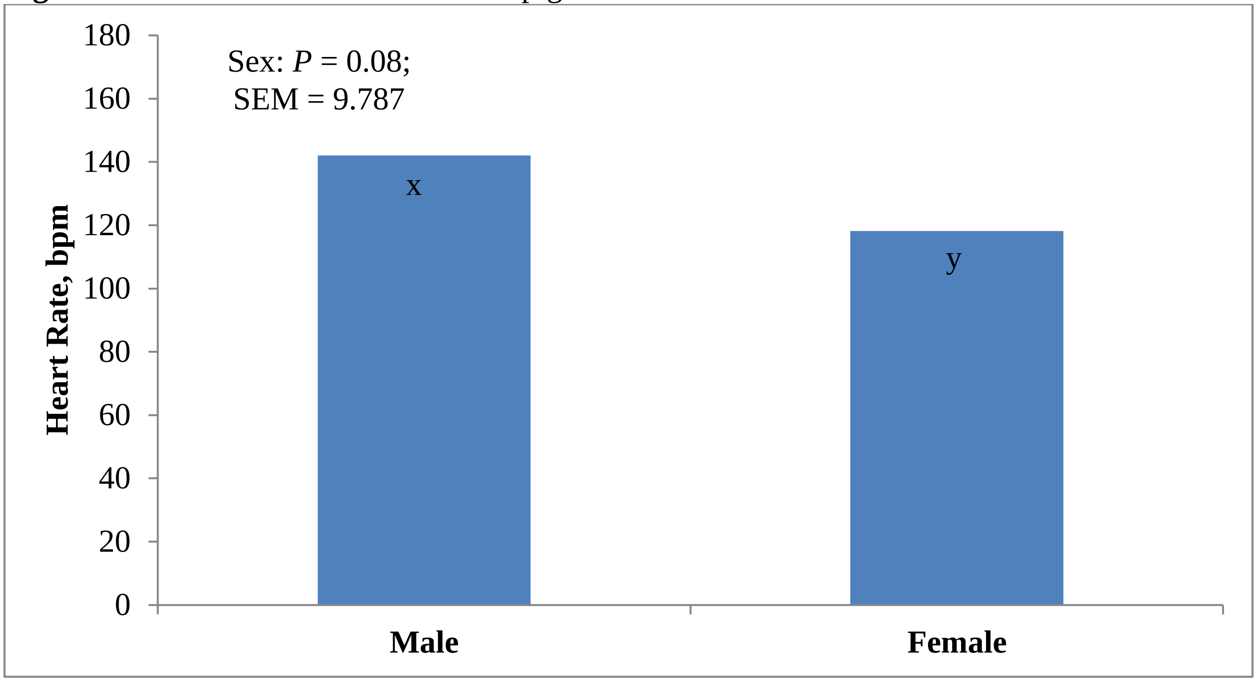
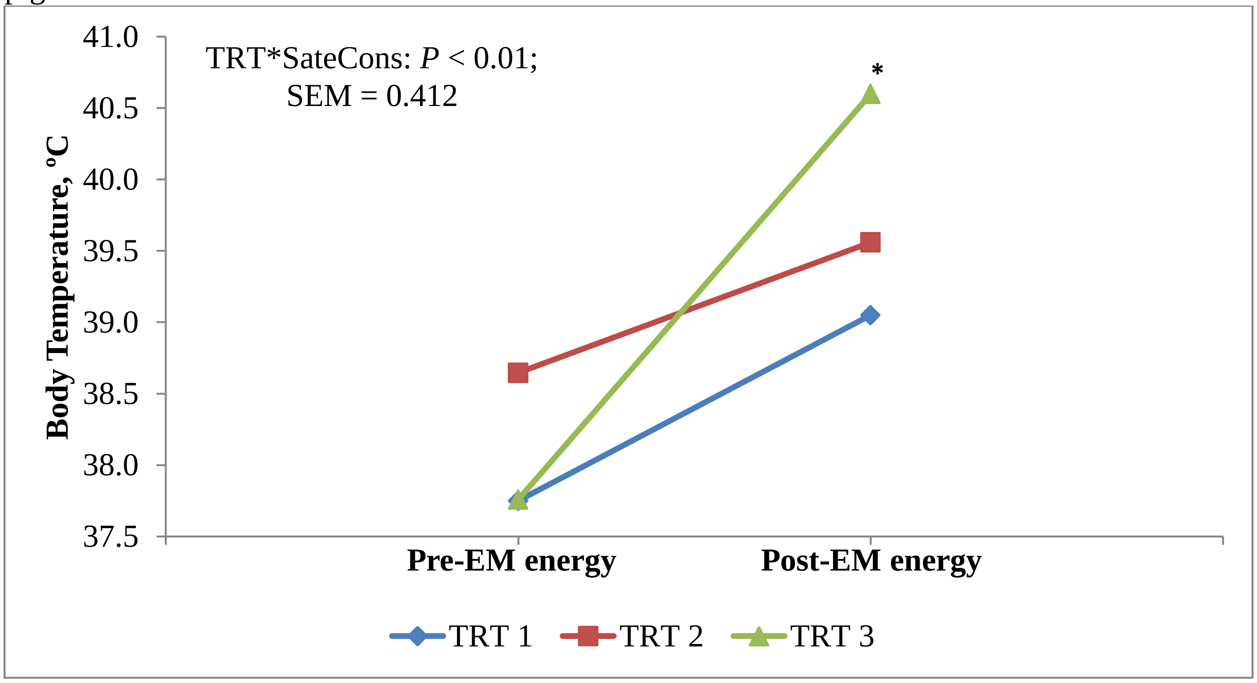
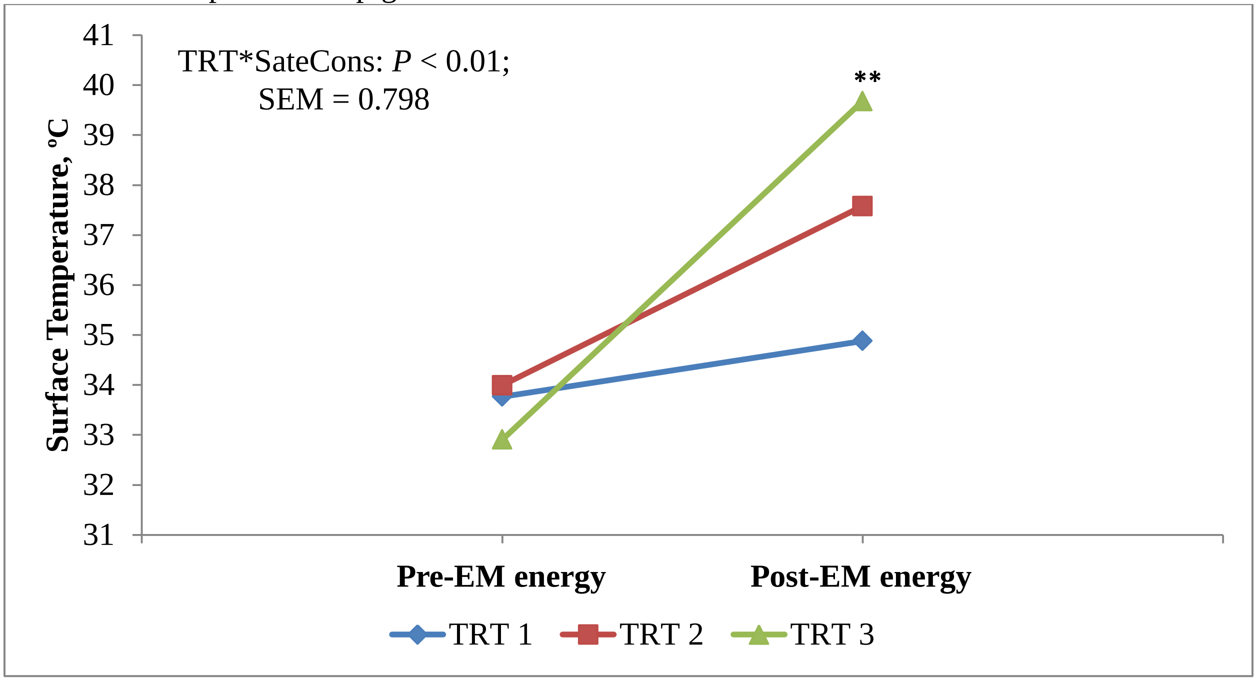


Figure 10. Effects of state of consciousness and treatment interaction on body temperature of piglets



***Indicates differences between treatments specifically after EM energy application (P = 0.05).

Figure 11. Effects of state of consciousness and treatment interaction on head surface temperature of piglets



** Indicates differences between treatments specifically after EM energy application ($P < 0.01$).

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