

EVALUATION OF HYPOBARIC HYPOXIA AS A LOW STRESS ALTERNATIVE  
TO CARBON DIOXIDE EUTHANASIA FOR USE WITH NURSERY PIGLETS

by

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B.S., Kansas State University, 2009

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2012

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## Abstract

Malnourished piglets that suffer from periweaning failure to thrive syndrome (PFTS) may show no signs of respiratory or enteric diseases but may have decreased feed intake and become debilitated after weaning. Euthanasia is a necessary component of swine production as it is sometimes the only option to alleviate suffering of piglets that are born with congenital defects or suffer from PFTS.

Fifty-eight nursery-aged piglets were utilized in two experiments to evaluate blood parameter differences between healthy and unthrifty piglets and to compare euthanasia methods. Piglets were categorized into two health groups: healthy or unthrifty. During selection, blood was collected for analysis of blood parameters. Piglets were euthanized 24-32 hours after initial blood sampling and a second sample was collected for comparison. After euthanasia, piglets were necropsied for evaluation of euthanasia on pulmonary lesions. No significant difference in number of pulmonary lesions was found between health groups ( $P = 0.88$ ). Healthy piglets had higher concentrations of glucose, ionized calcium and sodium, and greater  $p\text{CO}_2$  than unthrifty piglets ( $P \leq 0.05$ ). Unthrifty piglets showed higher concentrations of hemoglobin and hematocrit ( $P = 0.0002$ ) than healthy piglets.

Piglets were assigned to one of two euthanasia methods to compare electrophysiological and behavioral parameters of hypobaric hypoxia and carbon dioxide gas. Two piglets at a time were euthanized for each method. One animal in the pair was fitted with electrocardiogram and electroencephalogram monitoring devices during euthanasia. Behavioral parameters were also recorded. The average treatment times were  $27.4 \pm 6.7$  minutes for HH and  $13.8 \pm 5.1$  minutes for  $\text{CO}_2$ . Piglets euthanized via  $\text{CO}_2$  reached an isoelectric state faster than piglets euthanized via HH ( $P = 0.009$ ). Behavioral observations revealed gasping in 100% of  $\text{CO}_2$  euthanized piglets during the first five minutes of treatment and only 28.6% of HH euthanized piglets during the same period. During HH, 57.1% of piglets became ataxic in the first five minutes while 76.9% of  $\text{CO}_2$  piglets became ataxic during the same period. Results of this trial indicate that HH may be a lower stress alternative to  $\text{CO}_2$  as it causes fewer incidences of aversive behaviors in early stages of treatment.

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## Acknowledgments

Wow, I can't believe this journey is almost complete – I've planned and conducted the research, analyzed the data, written and defended the results and in a few short months it will be published in scientific journals. I wasn't sure I would ever get this far – earning my master's degree has by far been the most daunting, stressful, challenging, gut-wrenching, tear inducing and painful experience of my life. It's also been more rewarding than I can ever describe – the challenges I faced have transformed me into a better academic, professional, writer and scientist.

I first and foremost have to give glory to God. There are many times when my faith and my husband's support were the only things that got me out of bed in the morning to complete the journey. My grandparents taught me to pray in all situations and that mindset got me through this challenge – He kept me going.

That other fella I mentioned, Hyatt, who has for some reason decided to attach his wagon to mine for eternity, has been the most supportive, loving, gracious, and patient man on Earth. So many days I wanted to give up and quit, especially while in Australia. Hyatt, if not for you, I surely wouldn't have finished and would forever regret it. Thank you for your tough love, guidance, proofreading skills and support. You are my lobster.

To my parents, thank you for your support. I know you will probably always be confused about why I wanted to do research with hogs but you supported me nonetheless. To the rest of my family – thank you for understanding why I couldn't come home as often as I wanted. Thank you for your support and love. To the Frobose's – thank you for your kindness and support.

To my graduate committee and Dr. Terry Engle and Dr. Bernard Rollin– thank you for taking a chance on me and for your help over the past three years. Dr. Nelssen, your “Don't Quit” sign still hangs in my office and is a constant reminder that when things get tough, you've got to fight. Thank you from the bottom of my heart for listening to my worries and aspirations and always being positive.

I would be remiss if I didn't thank the friends who still love me even though I drove them crazy with my grad school stress – Rebecca (Tokach) Acheson, Martha Riese, Mandy Myers, Kelly Schulz, Natalie Miller, Melissa Navinsky, Michelle Colgan, Michelle Bartley, Lindsay Graber,

Jessie Vipham, Kristen Walker, Kristen Schulte, Kelsey Fuller, Jackie Hand, Mikki Nienhueser and probably some I forgot. You guys are awesome. There are no words to describe how much you all mean to me. Harold Melleion, thanks for your humor and Ninja Turtle style – you rock. I also want to thank the individuals who helped with my research projects – Colleen Hill, Scott Hill, Kyla Krissek, Megan Kulas, Melanie Peele, and Karen Sellins. Additionally, many thanks are given to Kent and Marian Condray who let me run a trial at their hog operation for four days with a large crew. Their hospitality is unmatched.



## CHAPTER ONE

### LITERATURE REVIEW

#### Stress:

Stress may occur in many forms, which may include external or internal stimuli acting on an animal and eliciting a physical, psychological or physiological response. Stress is not always detrimental to an animal because animals can experience good and bad stress. Good stress, or eustress, occurs when an animal is experiencing stress that may lead to beneficial outcomes to the body or does not harm the animal. Mating, exercise and play fighting are both occurrences that would cause an animal to experience eustress. Conversely, bad stress can be derived from a physical, emotional or psychological strain put on the animal. Bad stress can occur in many fashions such as aggressive handling, sickness and rough transport. These experiences may initiate the stress response, or 'fight or flight' response.

When an animal experiences stress, the sympathetic nervous system is activated. The 'fight-or-flight' response, is initiated when an animal must cope with emergency situations or when catecholamines such as norepinephrine and epinephrine are secreted. These catecholamines cause an increase in heart rate, constriction of blood vessels and dilation of air passages in preparation for the animal to defend itself or flee. Synthesis and secretion of cortisol occurs in response to activation of the sympathetic nervous system which then causes the fight or flight response. The animal's heart and respiratory rates accelerate, the pupils dilate to allow for improved peripheral vision, and in order to meet the needs of the muscles, capillaries become dilated to shunt blood to more important places. To achieve this increase in blood flow, blood vessels in the skin, gastrointestinal tract and kidneys constrict, thereby slowing the digestive, renal and reproductive systems. This shunting of blood increases the amount of blood flow to vital survival systems such as the respiratory and cardiac systems and skeletal muscles. These effects are produced partly by direct sympathetic nerve stimulation of target tissues and partly by the sympathetic

nerve-stimulated release of epinephrine and norepinephrine into the blood from the adrenal medulla (Reviewed by Colville and Bassert, 2002).

While the fight or flight response does prepare the body for fleeing from danger or fighting, acute stress can also have detrimental effects on the animal such as gastric ulcers and changes in immune function (Kelley, 1980). Weight gain and reproductive processes can be altered under stressful environments or situations (Hemsworth, et al. 1981a,b; Morrow-Tesch et al., 1994). Because of the widespread and immediate physiological response that occurs after stress, certain blood parameters may be altered that can be assessed and used to evaluate an animal's well-being and health (Barnett et al., 1991; Jensen-Waern and Nyberg, 1993; Hamilton, 2004; Nowak et al., 2007).

Cortisol:

Cortisol or glucocorticoid, a corticosteroid hormone, is secreted when an animal experiences stress. Cortisol secretion is controlled by the hypothalamic-pituitary-adrenal axis (HPA), which includes the pituitary gland, hypothalamus and the hypothalamic-sympathetic system which work together to provide peripheral control of stress responses. Cortisol secretion is activated by a stimulus, such as aggressive handling, which acts on one of the body's mechanoreceptors. In the case of aggressive handling, tactile or nociceptors would be activated. The stimulus creates a nerve impulse that travels to the paraventricular nucleus (PVN) of the hypothalamus. The nerve impulse causes the activation of the hypothalamo-pituitary axis and the release of catecholamines from the brain and adrenal medulla (Fulford and Harbuz, 2005; Levine, 2005). After activation, the HPA axis secretes corticotropin releasing factor (CRF) from the PVN via the median eminence of the hypothalamus and into the hypothalamic-hypophyseal portal system. The hypothalamic-hypophyseal portal system transports CRF from the hypothalamus to the anterior pituitary. Endocrine cells in the anterior pituitary gland react to the secretion of CRF by manufacturing and secreting adrenocorticotropin hormone (ACTH) and melanocyte stimulating hormone. Pituitary ACTH circulates through the blood and arrives at the adrenal cortex

where cortisol is secreted from the cells of the zona fasciculata. Once cortisol is circulating in the blood, it aids the body during stress by increasing glucose levels and suppressing immune system function through decreased lymphocyte production which in turn decreases inflammation.

The secretion of cortisol in response to stress in domestic farm animals has been evaluated repeatedly and is consistently used as a measure of stress in swine (McGlone et al., 1983; Westley and Kelley, 1984; Minton, 1994; Gevorkian et al., 1998; Hambrecht et al., 2004,2005; Chai et al., 2010). Cortisol is measured in a broad spectrum of activities such as pig handling, transport and exercise to determine the extent to which an animal is stressed (Worsaae and Schmidt, 1980; Jensen-Waern and Nyberg, 1993; Warriss et al., 1995; Hambrecht et al., 2005; Chai et al.,2010). Worsaae and Schmidt (1980) found increased cortisol levels in relation to the number of attempts needed to capture early weaned piglets, the more attempts that were needed for capturing the piglets, the higher the observed cortisol levels. Warriss et al. (1995) found increased ( $P < 0.001$ ) cortisol levels in pigs moved with sorting boards for two minutes prior to slaughter compared to pigs that were rested for three hours prior to slaughter. Hambrecht et al. (2005) reported increased levels of cortisol in pigs subjected to a high stress treatment (use of electric goads and loud yelling) prior to slaughter. Jensen-Waern and Nyberg (1993) utilized treadmills to induce eustress and found a significant increase in cortisol in pigs that were exercised on a treadmill for 3-5 minutes. Chai et al. (2010) compared three different variations of transport time and stocking density on 95 kilogram pigs. Results indicated that pigs transported for 5 hours exhibited greater increases in cortisol than pigs transported for shorter time periods of 30 minutes and 4 hours. Cortisol concentrations were also increased in pigs in the highest stocking density treatment. These data suggest that both the long transport time and high density are stressors that stimulate the hypothalamic-pituitary-adrenal axis (HPA) and the subsequent release of cortisol (Chai et al., 2010).

An advantageous characteristic of cortisol is that it can be measured via saliva therefore potentially reducing stress imposed on the animal that may be experienced during blood sampling (Chaloupkova et al., 2007; Couret et al., 2008; González et al., 2010). It is convenient to have more than

one option for cortisol sampling; however, it should be noted that cortisol concentrations vary depending on the medium being measured. Guzik et al. (2006) evaluated plasma and salivary cortisol in pigs that were fed supplemental tryptophan. Results showed that plasma cortisol levels ranged from 10.55 to 15.61  $\mu\text{g/dL}$  while salivary cortisol levels ranged from 2.00 to 2.55  $\mu\text{g/dL}$ . Koopmans et al. (2006) also supplemented tryptophan to piglet diets to evaluate its effects on neuroendocrinology, behavior and cortisol levels and, similarly to Guzik et al. (2006), salivary cortisol values were lower than plasma cortisol levels (values not indicated). Researchers should use cortisol sampling protocols that are comparable to similar studies in order to make comparing results more efficient and accurate.

In addition to variance between sampling methods, cortisol values also fluctuate between species. Differences between species can be seen when comparing salivary cortisol concentrations in beef to salivary cortisol concentrations in pigs. González et al. (2010) measured post-castration salivary cortisol in beef calves and found values ranging from approximately 0.6 to approximately 5.0 ng/mL. González et al. (2009) measured salivary cortisol in beef heifers that were delayed their feed rations for varying lengths of time. Maximum salivary cortisol concentrations ranged across treatments from 3.45 to 4.36 ng/mL. Koopmans et al. (2006) found mean salivary cortisol concentrations in pigs fed supplemental tryptophan ranging from approximately 0.9 ng/mL to approximately 3.7 ng/mL. As shown, the treatment the animal experiences can alter the value obtained. The variation of cortisol concentration across species could possibly be attributed to the size of the species, the genetic background or the surrounding environment of the animal.

An animal that has been exposed to a stressful experience or stimulus may not always elicit a strong cortisol response. Ritter et al. (2009) exposed market weight pigs ( $130.0 \pm 0.65$  kg) to various handling intensities, transport floor space and distances moved during handling. Results indicated that pigs exposed to aggressive handling and pigs moved 125 meters had greater values of blood lactate, blood partial pressure of carbon dioxide and rectal temperatures. However, no significant increase in blood cortisol was observed. These data may indicate that even though an animal is stressed because of

aggressive handling, cortisol may not be synthesized and released into the bloodstream. There may be a possibility of a stress response threshold that must be met to initiate the secretion of cortisol. Furthermore, the timing of sample collection may be important if cortisol is not secreted immediately after stress occurs.

#### Catecholamines:

The catecholamines epinephrine and norepinephrine are released from the adrenal medulla and postganglionic sympathetic nerves upon activation of the fight or flight response. Epinephrine and norepinephrine, which are hormones, can cause an increase in heart rate, contraction of blood vessels and dilation of air passages (Cannon, 1929) and prepare the animal to defend itself or flee as part of the fight or flight mechanism.

Biosynthesis of norepinephrine and epinephrine takes place in the adrenal medulla of the kidney. Norepinephrine is synthesized from tyrosine which is obtained either from the diet or the liver, and converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase. The synthesis cannot be reversed from this point on, as it is a non-reversible step. L-DOPA is then converted to dopamine via the aromatic amino acid decarboxylase. Dopamine itself may serve as a neurotransmitter within the brain and once it is synthesized dopamine  $\beta$ -hydroxylase catalyzes the conversion of dopamine into norepinephrine. To produce epinephrine, the chromaffin cells in the adrenal medulla secrete phenylethanolamine N-methyltransferase, which converts norepinephrine to epinephrine (Akers and Denbow, 2008). Norepinephrine synthesis may continue when tyrosine is made available from the diet or the liver.

The release of norepinephrine and epinephrine is activated by a stimulus, such as motion or pressure, which is applied to one of the body's receptors. The stimulus creates a nerve impulse that activates an action potential and travels to the hypothalamus. The nerve impulse is sent from the hypothalamus, which connects the nervous system to the endocrine system, to the preganglionic sympathetic nerve fibers. The nerve fibers innervate the adrenal medullary cells by releasing

acetylcholine from the neuron terminal. The acetylcholine binds to nicotinic cholinergic receptors on the adrenal medullary cells which then stimulate the secretion of catecholamines. Norepinephrine is generally more abundant than epinephrine (Sadock and Kaplan, 2007) because epinephrine increases become apparent only when physical stress is prolonged to the point of exhaustion (Terjung, 1979; Dehnhard and Claus, 1990).

Assessment of blood concentrations of catecholamines allows more precise study of physiological stressors such as integration of new pigs, weaning and pre-slaughter handling (Dantzer and Mormede, 1983). Catecholamines may have an undesirable effect on meat quality when present at low levels by causing increased glycogenolysis and glycolysis (Moss, 1984). Glycolysis converts glucose into pyruvate, which is then converted to lactate in the muscle. Post mortem glycolysis causes a decrease in muscle pH which contributes to pale, soft and exudative (PSE) pork. Lower levels of catecholamines have been found to reduce muscle glycolysis by reducing the second messenger 3',5'-cyclic adenosine monophosphate (cAMP) (Sutherland et al., 1965). Research conducted on how to reduce levels of catecholamines in stressed pigs has led to production practices that may improve meat quality (Adeola and Ball, 1992; D'Souza et al. 1998a, 1999; Lebret et al., 2006). Adeola and Ball (1992) suggest that supplementing dietary amino acids that are precursors to catecholamine synthesis could aid in reducing the stress response in pigs and ultimately, pale, soft and exudative pork. Through measuring epinephrine and norepinephrine, continual progress can be made towards alleviating stress in pigs and improving pork quality.

Assessment of catecholamine levels in the blood may be a useful, predictive indicator of the stress level of pigs and can be used to potentially develop lower stress production methods. Higher catecholamine levels have been seen in pigs exposed to high stress pre-slaughter handling when compared to pigs exposed to low-stress pre-slaughter handling (Hambrecht et al., 2004). Catecholamine levels also increase in pigs exercised on treadmills (Zhang et al., 1992) and pigs found to be subordinate to dominant pigs (Otten et al., 2002).

While there are advantages to using norepinephrine and epinephrine as markers of stress, there are difficulties associated with measuring catecholamines. These catecholamines are more difficult to quantify via a routine radioimmunoassay (RIA) or an enzyme-linked-immunosorbent serologic assay (ELISA) because those methods require a very specific antibody to detect the specific hormone of interest (either epinephrine or norepinephrine). Because norepinephrine and epinephrine differ structurally only by a side methyl group from their base amino acid, it is very hard to find an antibody that differentiates between the two. Due to small differences in molecular structure, plasma must be extracted and high pressure liquid chromatography (HPLC) must be used to separate the catecholamines before they can be analyzed with an RIA or ELISA. This process is more time consuming than a conventional RIA or ELISA used for cortisol or lactate.

#### Lactate:

Blood lactate has been utilized as a determinant of stress because pigs experience a rapid increase in blood lactate concentration when exposed to stressful handling procedures or environments (Benjamin et al., 2001, Hambrecht et al., 2004). Blood lactate concentrations vary depending on how the animal has been handled. Baseline lactate concentrations in catheterized, calm pigs vary from approximately 0.1 mmol/L (Neubert et al., 1996) to 1.9 mmol/L (Jensen-Waern and Nyberg, 1993). In contrast, Hamilton et al. (2004) reported lactate concentrations from 2.8 (for light weight pigs) to 3.1 mmol/L (for heavy weight pigs) after pigs had been restrained via snout snare. Blood lactate may increase even more dramatically if stress is prolonged or if handling is intense (Benjamin et al., 2001; Hambrecht et al., 2005). Benjamin et al. (2001) found blood lactate concentrations of 24 mmol/L in pigs that were subjected to aggressive handling over a distance of 300 meters and Hambrecht et al. (2005) found that pigs experiencing high stress handling prior to slaughter, such as handlers yelling and using electric goads, had blood lactate concentrations of 30.9 mmol/L.

Blood lactate concentration has been identified as a reliable determinant of stress in pigs (Benjamin et al., 2001; Ritter et al., 2008; Edwards, 2009, 2010). An increase in blood lactate occurs during stress because the animal has depleted its store of ATP derived from glucose and must produce more ATP, during which time lactate is produced as a by-product. Pyruvate, the end product of glycolysis, is converted into lactate by the enzyme lactate dehydrogenase (Horton et al., 2002). This final reaction is continually taking place, but the amount of pyruvate converted to lactate is increased when the amount of available oxygen is limited during intense exercise or activity (Tymoczko et al., 2010). After production, lactate can be oxidized within the muscle in which it was produced and transported to other muscles fibers for oxidation. Conversely, if lactate is not oxidized in the muscle, it is diffused from the muscle into the capillaries and is then carried in the blood to the liver (Brooks, 1986).

An advantage of utilizing blood lactate as a measure of stress is that blood lactate concentration increases quickly when an animal experiences stress. Benjamin et al. (2001) found that blood lactate concentrations in pigs subjected to aggressive handling while being moved through a 300 meter course increased from approximately 4 mmol/L to 25 mmol/L. These results indicate that lactate can be used as a stress indicator in a situation where the stressful experience isn't prolonged. Blood lactate concentrations can be measured quickly using a hand-held lactate analyzer in order to acquire dependable readings regarding stress in pigs (Edwards, 2009, 2010). Edwards et al. (2010) utilized a hand held lactate analyzer to collect data from pigs subjected to low stress loading and standard lairage, transport and handling procedures. Additionally, unpublished research by Buzzard et al. (2010) utilized the hand held lactate analyzer to obtain values from pigs that were restrained via snout snare or with sorting boards. The hand held lactate analyzer provides a reliable measurement of blood lactate concentration approximately 15 seconds after sampling and is practical for immediate, on-site measurement of blood lactate concentration.

While lactate is a reliable indicator of handling, transport and pre-slaughter stress, blood lactate concentration can be elevated during experiences that may not cause negative stress to the animal,



whereas aggressive handling would have negative effects on blood lactate concentration. For example, pigs exercised on treadmills for 3 minutes exhibited increased blood lactate concentrations during and immediately following the exercise treatment (0 minutes post- exercise, 10 minutes post-exercise) (Jensen-Waern and Nyberg, 1993).

When considering stress in pigs, it is important to avoid relying on a single metabolic parameter for measuring stress. If a researcher were to use only one metabolic parameter to evaluate stress and the method of analysis for that parameter could potentially fail or continually deliver errors. This would force the researcher to recollect samples or use another method of lab analysis, thus consuming more time and increasing the work load of the researcher. Measuring stress using different markers provides the researcher with more assurance that a value will be obtained.

Stressors:

Stressors are factors that can affect an animal's behavior and physiology. Environmental, physical and psychological stressors are factors that can influence an animal's behavior and occur throughout different phases of an animal's life. When an animal encounters a stressor, the stress response, also known as the fight or flight response, is initiated and provides the physiological response needed for the animal to defend itself or take flight. There are stressors in swine production across all stages from farrow to finish; however, for the purpose of this literature review the focus will be on stressors that affect piglets.

There are numerous challenges in an animal's environment that can activate the stress response (Salak-Johnson and McGlone, 2007). Environmental stressors such as dust, odor and noise affect piglets on all intensive pig farming operations and are "clearly irritating environmental agents whose complete absence, although impossible, would be preferable" (Scipioni, 2005). Krebs and McGlone (2004) concluded that various odors in a piglet's environment such as, isopropyl alcohol, maternal feces or the boar pheromone androstenone, can alter the behavior of the animal by causing it to avoid or move closer

to the source of the odor. This study shows that odor, an environmental stressor, affects a piglet's behavior and therefore may also cause stress. While environmental stressors are always present on a pig farming operation, producers should make effort to reduce their presence.

Thermal stress is another form of environmental stress which can create or intensify the effects on an animal and alter its internal functions. Additionally, thermal stress, as with all forms of stress, may impair disease resistance and directly reduce productivity (Curtis, 1981). Feed intake and growth rates are decreased when piglets are subjected to temperatures 10° Celsius outside of the thermoneutral zone (TNZ), which is the zone where heat production remains basal, maximum comfort is experienced and where an animal experiences optimum health and performance (Ames, 1980). Morrow-Tesch et al. (1994) found that piglets that experienced heat and social stress had lower numbers of monocytes, thereby decreasing the piglets' immunity. Sutherland et al. (2007) evaluated the effects of heat stress and social rank on porcine reproductive and respiratory syndrome (PRRS) positive piglets and healthy piglets. PRRS positive piglets that were heat stressed exhibited suppressed levels of plasma cortisol which suggests that heat stress may modify the immune response to PRRS. Additionally, chronically heat-stressed pigs showed reduced cortisol and adrenocorticotropic hormone (ACTH). Results from Sutherland et al. (2007) suggest that decreased cortisol and ACTH concentrations in response to heat stress may be due to reduced adrenal responsiveness to ACTH.

Commingling, weaning and social stress are examples of psychological stressors. When piglets are weaned, or taken from their mother at approximately 2-4 weeks of age, they experience stress. This stress stems from the abrupt nutritional, social and environmental changes associated with weaning (Niekamp et al., 2007). Weaning involves a change in diet from the sow's milk to solid ration, a new habitat, separation from the sow and sometimes mixing with conspecifics (Weary et al., 2008). A piglet undergoing weaning stress may experience decreased immunity (Niekamp et al., 2007), depressed feed intake and decreased intestinal villi (Pluske et al., 1997). Research conducted in an effort to decrease weaning stress has discovered practices that may improve piglet well-being (Bruininx et al., 2002;

Koopmans et al., 2006; Niekamp et al., 2007). Niekamp et al. (2007) exposed different aged weaning groups (14, 21 or 28 days of age) to long or short photoperiods to examine the effects on cortisol and growth. Results showed that cortisol was lower in piglets exposed to long day treatment than in short day treatment across all three age groups. Additionally, piglets on the long day treatment had greater average daily gain (ADG) than short day piglets and the 28 day old weaning group in the long day treatment had the greatest body weight relative to all other treatment groups. Prolonging weaning until piglets are approximately 28 days of age may contribute to lower stress during weaning. Bruininx et al. (2002) offered a commercial creep feed diet to suckling pigs and found that piglets who consumed creep feed prior to preweaning started eating more quickly post weaning than did piglets who did not consume creep feed before being weaned. Koopmans et al (2006) supplemented L-tryptophan to weaned piglets in order to evaluate the effects of L-tryptophan on intestinal integrity. Results indicated that intestinal villi were more pronounced in piglets supplemented L-tryptophan than piglets not supplemented. Feeding strategies may provide an opportunity for producers to decrease the stress associated with weaning.

Commingling is another form of psychological stress. When pigs first meet, they go through a period in which they establish a social hierarchy (Grandin, 1989). During this time some pigs may fight and others may submit to the dominant pigs. Pigs that were weaned at varying ages and then commingled had no difference in the amount of time engaged in aggressive behavior between age groups; however, the younger pigs were slower to habituate to new environments than older pigs (Davis et al. 2006).

Research conducted by Jensen and Yngvesson (1998) provides insight to behaviors exhibited by commingled pigs such as biting, nose to nose contact and tilting of the pig's head while retreating. Jensen and Yngvesson (1998) observed that mutual nose contact almost always occurred prior to a fight and in agreement with their hypothesis, larger pigs were considerably more likely to win. The experimenters concluded that pigs fought in order to assess the fighting ability of their pen mates. As a result of trials such as Davis et al. (2006) and Jensen and Yngvesson (1998), it may be difficult for producers to develop production methods to prevent fighting during commingling.

Restraint and handling are forms of physical stressors that occur regularly during processing, transportation and veterinary treatment. Improper handling can give rise to detrimental effects to piglet well-being and health (Grandin, 1986). To reduce the amount of stress experienced, pigs should be handled and restrained for the least amount of time possible. Panepinto et al. (1983) developed a portable restraint system that suspends the pig in a comfortable cotton sling and results showed that pigs will lie quietly during most experimental procedures. Damm et al. (2000) evaluated a simple non-surgical technique for collecting continuous blood samples that doesn't require repetitive restraint of the pig. Matte (1999) and Baldi et al (1989) collected blood samples from the jugular vein while piglets were in dorsal recumbence. The dorsal recumbency restraint method allows for access to the vein while decreasing the possibilities of injury to the pig.

High stress handling can alter meat quality (Warriss et al., 1995; D'Souza et al., 1998; Hambrecht et al., 2004,2005b). D'Souza et al., 1998 found pigs that had been negatively handled before slaughter had lower muscle pH than pigs that were handled normally before slaughter. Hambrecht et al. (2005b) exposed pigs to high stress preslaughter handling that consisted of being moved with electric goads while workers were yelling loudly. High stress handling pigs had decreased muscle glycogen and darker colored pork when compared to meat from pigs that underwent low stress preslaughter handling.

#### Euthanasia:

Some stressors are so detrimental to the pig's health and well-being that the animal must be humanely euthanized in order to prevent further suffering and pain. Additionally, pigs that have a congenital illness that has rendered them terminally unhealthy, such as porcine respiratory and reproductive syndrome, or who are too small to survive are often euthanized. These pigs are sometimes called "unthrifty" and would experience pain and suffering throughout their life if not euthanized. Morrow et al. (2010) stated that 86.6% of surveyed farm employees think it is more humane to euthanize a sick pig that appears to be in pain than to let it suffer and die naturally in its environment.

Euthanasia provides a means for a painless death and is performed when the animal is experiencing pain, distress or suffering exceeding levels pre-designated by the production facility (Working Party Report, 1997). Euthanasia should meet criteria and objectives that have been determined humane. The chosen method of euthanasia should require minimum restraint, be reproducible, minimize fear and stress, cause a rapid and painless death and be aesthetically tolerable to the handler (Working Party Report, 1997).

Handler training is very important to any method of euthanasia. During euthanasia, an animal may exhibit signs of distress or pain. Animals in pain during euthanasia may salivate, urinate or defecate, experience tachycardia, emit distress calls or attempt to escape the procedure. It is vital for the handler to be knowledgeable of such signs so that if necessary, an alternative form of euthanasia can be used to terminate the animal and alleviate its pain. Handlers must also be trained to recognize and confirm death of the animal. If a method of humane euthanasia is performed incorrectly and causes pain or distress, that method is no longer humane.

Handlers must be acceptant of the sight of an animal being euthanized. Morrow et al. (2010) determined that 45.7% of farm employees do not like to hold the pig while euthanasia is performed. In this case, it is important for farm owners to train employees to perform other methods of euthanasia that don't require the employee to hold the animal. The employees surveyed in Morrow et al. (2010) appeared to be more concerned with the welfare of the pig than in any certain method of euthanasia. Results of this survey point out the need for alternative methods of euthanasia to be developed and practiced on farms in order to meet both animal welfare standards and the ethical standards of farm employees.

There are several methods of on-farm euthanasia currently being utilized in the swine production industry. Cervical dislocation, otherwise known as "knocking" or blunt force trauma, is the chosen method of on-farm swine euthanasia in approximately 93.6% of surveyed farms (Morrow et al., 2010). Knocking is performed by a handler grasping a piglet by the hind legs, and then forcefully striking the

forehead of the pig on a hard surface, usually a concrete floor. This method is effective at euthanizing the piglet instantly if performed accurately by a trained employee or handler. Knocking is considered humane if carried out by trained personnel and death is confirmed by destruction of the brain (Working Party Report, 1997). However, in cases where the pig isn't terminated after the first trauma to the head, the procedure must be done more than once, thereby decreasing its efficiency and causing the animal to experience pain. The pain a pig experiences during this procedure has yet to be determined.

Captive bolt guns are used to render an animal unconscious immediately prior to exsanguination. The bolt, usually made of stainless steel, is propelled forward by compressed air and penetrates the skull inducing immediate unconsciousness. This method is used to euthanize sows and sometimes finisher pigs and is the most acceptable physical method of euthanasia for large mammals (Working Party Report, 1997). It is not recommended that a captive bolt be used on a pig less than 12 pounds (AASV, 2008; OIE, 2009) and it is preferred that a free bullet be utilized to increase operator safety (Working Party Report, 1997). As with all euthanasia methods, operators must be well trained in order to ensure correct shot placement of the bullet (Universities Federation for Animal Welfare, 1989). Extra attention should be given to the maintenance of the captive bolt gun to ensure that the correct bullet is used and that the gun is in proper working order for every use. Because the captive bolt gun is operated by humans, a certain amount of error is to be expected in the form of misplaced shots. If the shot is misplaced, the animal could experience painful trauma and could potentially be angered and attempt to harm the operator. Additionally, this method can only accommodate one pig at a time and therefore is not as time efficient as gas stunning when euthanizing several pigs is necessary.

Euthanasia by means of an injection of sodium pentobarbital is a widely accepted method of on-farm euthanasia. Sodium pentobarbital can be administered intravenously, intramuscularly, intrarenally, intraperitoneally or intrathoracically; however, intravenous administration is the quickest method and causes the least distress to both the handler and the animal (Lucke, 1979; Working Party Report, 1996). Sodium pentobarbital euthanizes an animal by depressing the central nervous system therefore causing

respiratory and cardiac arrest. This method is effective at rapidly euthanizing the animal, therefore increasing its attractiveness to commercial swine producers looking for a simple, safe method of euthanasia.

While injection of sodium pentobarbital is a rapid and effective method of euthanasia, there are disadvantages. Sodium pentobarbital poses danger to handlers due to its lethal capabilities. If an animal is nervous or exhibiting movement while being restrained, the handler could unintentionally stick him or herself with the injection needle and might possibly need medical attention. Additionally, sodium pentobarbital is a controlled substance and therefore, only a licensed veterinarian is legally allowed to use sodium pentobarbital as a form of euthanasia (Irwin, 2010). Because of this regulation, using sodium pentobarbital as a method of on-farm euthanasia is difficult unless the producer employs a veterinarian to be on-farm at all times. Employing a full time veterinarian may not be feasible to a small production facility and adds to the overhead costs that a producer incurs. Therefore, sodium pentobarbital may not be the best euthanasia option for small swine farms.

The use of carbon dioxide as a means of on-farm euthanasia has potential to be adopted as a primary method of euthanasia in the swine industry. Carbon dioxide gas has been investigated in experimental trials in order to determine whether it meets animal welfare standards for use as an anesthetic (Kohler et al., 1998) and CO<sub>2</sub> has been compared to electrical stunning in abattoirs as a method of pre-slaughter stunning (Velarde et al., 2000). Gerritzen et al., (2000) evaluated the behaviors broilers exhibited when exposed to varying concentrations of CO<sub>2</sub> and found that broilers avoided or could detect increased levels of CO<sub>2</sub>.

There are advantages to adopting carbon dioxide euthanasia. This method requires less handling of the animal than cervical dislocation or captive bolt and allows more animals to be euthanized at one time (Nowak et al., 2007). Although the animal may be rendered unconscious with the gas, death must be achieved by means of exsanguination (Working Party Report, 1997). In the European Union, carbon

dioxide concentration of 70% has been determined acceptable; however the Scientific Veterinary Committee (ScVC) has proposed a concentration of at least 80% be used during euthanasia (ScVC, 1997). Holst (2001) stated that when using carbon dioxide as a form of euthanasia, there should be no spontaneous blinking of the eye, no convulsions and the pig should only experience brief gagging and gasping. The demands set forth by Holst (2001) can be most correctly met when pigs are stunned with 90% (88-91%) carbon dioxide for 100 seconds (Nowak et al., 2007).

Some humane issues have been raised regarding the use of carbon dioxide as a form of euthanasia. Studies have reported that pigs exposed to high concentrations of CO<sub>2</sub> exhibited behaviors that suggest the animals were uncomfortable and experienced adverse effects caused by the gas (breathlessness, avoidance of the gas, convulsions, coughing, sneezing) (Mullenax and Dougherty, 1963; Raj and Gregory, 1995, 1996). Deiss et al (2006) reported that eight out of ten pigs immersed in 80% carbon dioxide exhibited violent jumps, respiratory difficulties and vocalizations. Raj and Gregory (1995) evaluated the behaviors of pigs that were presented the option of eating in a 90% Argon environment or leaving the area. Those behaviors were compared to the behaviors of the same pigs given the option to eat in a 90% CO<sub>2</sub> environment or leave the area. Results indicated that pigs avoided the CO<sub>2</sub> environment even if they had been fasted for up to 24 hours.

While CO<sub>2</sub> euthanasia is not currently a primary method of on-farm euthanasia, it is widely used in slaughter plants in the United States, Australia, Denmark and several other countries. Pigs are loaded onto a ferris-wheel type gondola which submerges pigs to an environment of approximately 90% CO<sub>2</sub>. Hartung *et al.* (2002) determined that an environment of 90% CO<sub>2</sub> works most effectively to render pigs insensible after approximately 70 seconds. This method of stunning is effective, however, special attention must be paid to the mechanics of the process to ensure that the concentration stays at appropriate levels. Additionally, line maintenance in the plant is crucial for the welfare of pigs; if the line stops or there is a breakdown, pigs could begin to regain sensibility. Therefore, a separate line that is not affected by the fabrication floor is ideal.



Because of the varied opinions about the well-being of pigs that are stunned or euthanized with carbon dioxide, there is a need for development of a method of euthanasia that does not provoke aversive behaviors or harmful side effects such as convulsions or respiratory difficulty.

The utilization of hypobaric hypoxia, which is the deprivation of oxygen at less than normal air pressure, may potentially serve as a lower stress method of euthanasia than current methods of on-farm euthanasia such as carbon dioxide euthanasia, cervical dislocation and captive bolt. The process is comprised of two events, hypobaria and hypoxia, that can exist independently or in this case, simultaneously. Hypobaric hypoxia occurs when atmospheric partial pressure of oxygen is less than the physiological level necessary to maintain proper oxygenation of body tissues (Booth, 1978). Due to the low level of oxygen, the electron accumulation thereby attacks the ground state of available oxygen and forms a superoxide anion ( $O_2^-$ ) which starts a chain reaction to form hydrogen peroxide and hydroxyl radicals that attack the membrane lipid. This chain of events decreases the activity of cellular defense systems such as antioxidant enzymes (Maiti et al., 2006). Side effects of this physiological state range in variety and strength. Van Liere (1963) stated, "A person exposed to a low oxygen tension often passes through an initial stage of euphoria, accompanied by a feeling of self-satisfaction and a sense of power. The oxygen want stimulates the central nervous system so that the subject may become hilarious and sing or shout, and other emotional disturbances may manifest themselves." The length of exposure to hypobaric hypoxia is a strong determinant of the intensity of the side effects. As exposure duration increases, so does the intensity of the effects.

Hypobaric hypoxia euthanasia is performed using a vacuum chamber which is very similar structurally to chambers used in carbon dioxide euthanasia. The vacuum chamber is large enough to accommodate nursery weight pigs comfortably but small enough to be portable. A closed loop automated control system is used to decompress the vacuum chamber so that ascension to the desired altitude occurs at a predetermined and gradual rate. Additionally, there is no air supply to the chamber. While the

pressure inside of the chamber is decreasing, the amount of oxygen is also decreasing. The combination of these different yet simultaneous effects causes the animal to undergo hypobaric hypoxia.

Hypobaric hypoxia has been studied as early as the 1950's by the aerospace and aeronautics industries in order to determine how astronauts and pilots would be affected when subjected to high altitudes and low partial pressures of oxygen (Cole et al., 1953; Bancroft and Dunn, 1965; Dunn et al., 1965; Cooke and Bancroft, 1966). Laboratory animals have been used in studies in order to understand the physiology and pathology of high altitude diseases such as high altitude pulmonary edema (HAPE), high altitude cerebral edema (HACE) and hypobaric hypoxia (Bancroft and Dunn, 1965; Viswanathan et al. 1969; Heath, 1992; Stepanek et al., 1998). Bancroft and Dunn (1965) experimented with dogs to evaluate the effects of rapid decompression on animal behavior and lung function. Results indicated lung lesions which were attributed to rapid decompression and agreed with results from Edelman and Hitchcock (1952), who rapidly decompressed dogs from approximately ground level altitudes to >50,000 feet in 0.012 seconds. Stepanek et al. (1998) subjected 4-10 week old piglets to gradual decompression of approximately 454.5 meters per minute (1491 feet per minute) for varying amounts of time ranging from 12-72 hours. Results showed that all animals exhibited lethargy and abnormal breathing, and subsequent necropsy revealed no damage to the central nervous system, heart or lungs.

The physiology of a pig is very similar to a human's in many aspects: pigs are omnivores and therefore have similar metabolism and digestive systems. Additionally, the cardiac and pulmonary systems are so similar that pigs are currently used as organ donors in xenotransplantations for tissues such as heart valves. These characteristics qualify the piglet as an acceptable model for comparable human research studies.

There has not been extensive research conducted on hypobaric hypoxia euthanasia in pigs. Findings from previous experiments performed on rats and dogs found decompression to be painful and stressful, therefore in many states decompression cannot be used as a method of euthanasia (Dunn et al.,

1965; Wis. Stat. Ann. 951.025. 1985; Nakanishi et al., 1995; Working Party Report, 1997; American Humane Association, 2010). Previous research using decompression, which is a term that can be used interchangeably with hypobaric hypoxia, was not performed using precise control of the hypobaric chamber. However, MacGregor (2008) used a slow decompression rate with a vacuum chamber that was controlled by an automated system. The development of an automated system allows for the precise control of the rate of ascension and thus gives more precise information as to how the rate at which animals are euthanized may ultimately affect their well-being.

On-farm euthanasia is a critical part of swine production. If a piglet is to be euthanized, the operator should strive to utilize a low-stress and painless procedure. It has been determined that carbon dioxide euthanasia is uncomfortable and causes adverse effects on the animal (Mullenax and Dougherty, 1963; Raj and Gregory, 1995, 1996). Additionally, determining the pain associated with cervical dislocation is subjective and difficult to quantify. Thus there remains a need for a low-stress, painless procedure for on-farm euthanasia. Hypobaric hypoxia may potentially replace other methods of on-farm euthanasia which have been deemed stressful or painful.

Swine producers must strive to implement low-stress handling into their production practices in order to avoid causing adverse effects of stress on their animals. The improper handling of pigs can cause stress, which can yield detrimental effects on animal well-being and physiological functions (Grandin, 1986) such as growth and reproductive function (Hemsworth, et al. 1981a, b, Von Borell et al., 2007) and meat quality (Warriss et al., 1995; D'Souza et al., 1998b; Hambrecht et al., 2004, 2005; Chai et al., 2010; Edwards, 2010). Stress can also alter blood parameters used to evaluate animal well-being and health (Barnett et al., 1991; Jensen-Waern and Nyberg, 1993; Hamilton, 2004; Nowak et al., 2007). Adverse effects caused by stress may be prevented or reduced if producers employ low-stress science based production practices.

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## CHAPTER TWO

# EVALUATION OF BLOOD PARAMETERS AS AN EARLY ASSESSMENT OF HEALTH STATUS IN NURSERY PIGS

### Summary

Piglets were categorized as healthy or unthrifty, and significant differences in certain blood gases and some ion concentrations were observed between health groups. However, differences between healthy and unthrifty pigs were not apparent upon necropsy. Assessment of hematological indicators may be a useful tool in monitoring health of nursery piglets.

Keywords: swine, blood, euthanasia, necropsy

### Introduction

Unthrifty piglets, which may account for approximately 1% of a nursery group,<sup>1</sup> may be weak, malnourished or dull<sup>2</sup> and may be more susceptible to diseases such as PRRSV or *Streptococcus suis*. Additionally, lightweight or malnourished piglets may also suffer from periweaning failure to thrive syndrome (PFTS) and therefore may show no signs of respiratory, systemic or enteric diseases but may have decreased feed intake and become increasingly debilitated after weaning.<sup>3</sup> Some piglets may suffer from other wasting diseases that cause loss of appetite, fever, dyspnea and weight loss. Blood sampling for the evaluation of hematological indicators can be highly valuable in the diagnosis, treatment and/or prognosis of many diseases<sup>4</sup>. Additionally, monitoring herd health via hematological indicators may potentially reveal adverse conditions even though the animal may not be displaying apparent clinical signs of disease.<sup>5</sup> Therefore, routine diagnostic sampling and evaluation of hematological indicators may aid in early identification of disease or disease susceptible piglets, which may prevent death loss from infection.

A deviation from normal hematological values can give an indication of how the piglet's environment is affecting the animal's physiology<sup>5</sup> and can aid producers in better herd and health management. Therefore, the objective of this study was to determine if hematological indicators may be used to identify disease susceptible (e.g. unthrifty) piglets.

## Materials and Methods

All animal use, handling and sampling techniques described herein were approved by the Kansas State University Animal Care and Use Committee.

Fifty eight piglets (22 barrows and 36 gilts; Danbred × PIC 327, USA, Hendersonville, TN) with an average weight of  $5.6 \pm 1.3$  kg were utilized in this experiment. Weaning occurred on d 21 and piglets were approximately 21 to 28 days old during the study period. Piglets were housed in environmentally controlled nursery barns in 2.74 m x 3.05 m pens. Each pen contained approximately 28 piglets and contained a single cup waterer and one double-sided feeder. Piglets consumed a corn, grain sorghum and soybean meal-based diet. A veterinarian assigned piglets to two health categories: healthy or unthrifty. Unthrifty piglets were free from abscesses or injury and were characterized through visible indicators of sickness, including but not limited to: coughing, apparent weakness, distended abdomen, apparent dehydration or malnourishment, lethargy and emaciation. Healthy piglets were those that did not exhibit any of the previous signs within the same groups.

### *Blood Sampling*

Piglets were selected randomly for blood sampling on one of three different days, designated as Day 1 (n = 20), Day 2 (n = 22) or Day 3 (n = 16), and piglets were euthanized approximately 24-32 hours after blood sampling. Approximately 15 ml of blood were collected into two tubes from each piglet via jugular venipuncture: 1) 9 ml K<sub>3</sub>EDTA tube (#22-040-037 Fisher Scientific, Pittsburgh, PA) for analysis of stress hormones and 2) 6 ml lithium heparin tube (#02-687-97 Fisher Scientific, Pittsburgh, PA) to be used for analysis of blood gases and ion concentrations. After blood sampling, piglets were weighed, ear tagged and marked with a livestock grease marker.

Prior to being centrifuged, a subsample of the blood collected into the lithium heparin tube was used for analysis of lactate concentration (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany), and glucose, ionized calcium (iCa), potassium, sodium, hemoglobin, hematocrit, pH and partial pressures

of oxygen ( $pO_2$ ) and carbon dioxide ( $pCO_2$ ) (iSTAT with CG8+ cartridge, #600-9001-25, Abaxis, Union City, CA).

Blood samples were centrifuged on-site for 15 min at 1000 x  $g$  at room temperature and the resulting plasma was removed, transferred to a polypropylene storage tube and stored on ice during transport to the lab. Plasma samples were stored at  $-20^\circ\text{C}$  until analysis of cortisol and catecholamines.

Plasma cortisol was analyzed using a COAT-A-COUNT Kit (#TKC01, Diagnostic Products Corporation, Los Angeles, CA) and a Packard Cobra Gamma Counter (PerkinElmer, Waltham, MA). Recovery for the cortisol assay was 100.14% and parallelism was 90.45%.

Plasma epinephrine and norepinephrine were isolated using activated alumina and 0.1M  $\text{HClO}_4$  and quantified in duplicate using HPLC as described by Holladay and Edens.<sup>6</sup> A plasma sample of 0.5 ml was combined with 250 ng of the internal standard; 3,4-dihydroxybenzylamine hydrobromide (DHBA). Catecholamine:DHBA peak height ratios for samples and standards were determined and sample catecholamine concentrations were calculated using the regression equation generated from each catecholamine standard. Recovery of the internal standard ranged from 83-87% and duplicate samples were averaged when coefficients of variation (CV) were less than or equal to 5%. Duplicate samples with CV greater than 5% were re-analyzed until variation was within the acceptable limits.

#### *Euthanasia & Necropsy.*

Piglets were euthanized two at a time, in one of two ways: 1) slow ascent hypobaric hypoxia (HH) (approximately 36.9 m/s) or 2) carbon dioxide gas ( $\text{CO}_2$ ) (induction of approximately 20% of the chamber volume ( $1.0 \text{ m}^3$ )/minute). Approximately 3 min after cessation of cardiac and brain electrical activity (as monitored via ECG and EEG, respectively) necropsies were performed by a certified veterinary pathologist to evaluate the effect of the two euthanasia methods on the presence of pulmonary lesions. Only pulmonary lesions were recorded for this trial. After necropsy, piglets were classified into three categories: pigs with pre-existing lesions (gross evidence of disease that was not related to euthanasia method), pigs with significant pulmonary lesions (related to the euthanasia process, localized

in the lungs, excluding cyanosis) and pigs with no significant lesions. For the purposes of this paper, we will be discussing pre-existing lesions and the relationship to health status.

#### *Statistical Analysis.*

Blood hormone, gas and ion concentration data were analyzed using PROC MIXED in a completely randomized design in SAS 8.2 (SAS Institute Inc., Cary, NC) with the health status serving as the fixed effect. Day was initially used as a blocking parameter in the statistical analysis of blood parameters and no block interactions were observed so it was removed from the analysis. The Kendwardroger approximation was used to calculate denominator degrees of freedom. Incidences of pulmonary lesions were analyzed using the PROC FREQ CHISQ function. Pig was the random effect in all analyses. Piglets were euthanized in the chamber two at a time; each use of the euthanasia chamber, signified as 'run', was the experimental unit.

#### **Results**

Healthy piglets had higher concentrations of glucose, ionized calcium and sodium, and greater  $p\text{CO}_2$  than unthrifty piglets ( $P \leq 0.05$ ). Unthrifty piglets showed higher concentrations of hemoglobin and higher hematocrit ( $P = 0.0002$ ) than healthy piglets. No significant differences were detected in epinephrine, norepinephrine, cortisol, potassium, lactate, pH or  $p\text{O}_2$  ( $P \geq 0.34$ ) between healthy and unthrifty piglets. Values for blood parameters are reported in Table 1.

Grossly observable pulmonary lesions that could be attributed to health status and not euthanasia method were observed in one healthy piglet and two unthrifty piglets. These lesions were abnormal for any pig of any stage of life or health status and it was determined that the lesions were not caused by euthanasia method. No significant difference in number of pulmonary lesions was found between health status groups of piglets ( $P = 0.88$ ). A more detailed analysis of the comparison between different methods of euthanasia was performed, and those results are beyond the scope of the current report.

#### **Discussion**

The blood parameters measured in the present experiment included markers for stress, energy or nutritional status and blood composition. Alterations in these parameters may indicate a disruption in



homeostasis and therefore closely monitoring their levels may aid in the early detection of diseases or conditions that either may not show clinical signs in piglets or may simply lead to unthriftiness.

Results from this trial seem to indicate that unthrifty piglets may have been dehydrated or malnourished, yet the parameters assessed do not directly provide information as to why unthrifty piglets may not have been eating or drinking. Anderson et al.<sup>7</sup> found normal hematocrit values in five day-old weaned miniature pigs to range from 29.8% -32.8%. In the present study, unthrifty piglets had an average hematocrit value of 35%, which was significantly higher than that of healthy piglets. Higher than normal hematocrit can influence cardiac function,<sup>8</sup> and may be evidence of dehydration or anorexia. For example, Xin et al.<sup>9</sup> fasted piglets for 72 hours and found that hematocrit values started to increase at the onset of the fasting period and continued to increase throughout the fasting period (33.5% – 40.1%). By comparison, the present results may indicate that pigs categorized as unthrifty were fairly early in the “fasted” stage, because their average hematocrit of 35% is at the lower end of the range found by Xin et al.<sup>9</sup>

Biologically adequate levels of hemoglobin in 21-28 day old piglets are reported to range from 9.2 – 10.5 g/dL.<sup>10</sup> In this study, unthrifty piglets had hemoglobin levels of 11.7 g/dL, while healthy piglets had significantly lower hemoglobin values of 9.9 g/dL. Healthy piglets had hemoglobin values within the biologically normal range and the greater hemoglobin values of the unthrifty piglets may be attributed to dehydration or malnourishment.

Our results show that unthrifty piglets exhibited lower concentrations of glucose than healthy piglets, which may be an additional indicator of depressed feed intake and/or dehydration. Normal blood glucose concentrations for piglets range from 65-95g/dL.<sup>11</sup> Unthrifty piglets in this study exhibited glucose concentrations of 78 mg/dL, which is within the healthy range but lower than that of their healthy contemporaries (95 mg/dL). The present results parallel those of Gentz et al.<sup>12</sup> who fasted piglets for up to 120 hours and found that blood glucose concentrations in newborn, 1, 3, 9 and 16 day old piglets decreased over the treatment period (values not given).

Biologically normal serum concentrations of sodium in three week old piglets are  $144.9 \pm 2$  mmol/L.<sup>13</sup> In this study, unthrifty piglets showed lower than normal sodium concentrations of 134 mmol/L, as did healthy piglets at 137 mmol/L. Accensi et al.<sup>15</sup> fed rations containing increasing levels (280, 560 and 840  $\mu\text{g}/\text{kg}$ ) of deoxynivalenol (DON), a mycotoxin found in cereal grains such as wheat, barley and corn, to three different groups of piglets and found that sodium values for the three groups of piglets fed DON (142.8, 142.8, 142.2 mmol/L, respectively) did not vary throughout any treatment group and were not significantly different than piglets on the control diet (142.9 mmol/L). Results from Accensi et al.<sup>15</sup> indicate that although feed intake is decreased, sodium levels did not necessarily decrease. Other hematological factors in the present study indicate that pigs categorized as unthrifty were likely dehydrated and/or malnourished. However, taken together with the results of Accensi et al.<sup>15</sup> described above, it is possible that some other factor may be responsible for the reduced sodium levels seen here.

Normal blood serum concentrations of ionized or free calcium (iCa) for 14-35 day old piglets fall between 10.9 mg/dL (2.7 mmol/L) and approximately 11.5 mg/dL (2.9 mmol/L)<sup>13</sup>. In this study, iCa concentrations of both healthy and unthrifty piglets were below what is considered normal (1.36 and 1.30 mmol/L, respectively). Our results show lower calcium concentrations than Tuscherer et al.,<sup>16</sup> who found that piglets who died within 10 days of birth had higher ( $P = 0.04$ ) calcium values (3.02 mmol/L) than piglets who lived 10 or more days post-birth (2.95 mmol/L).

Normal partial pressures of carbon dioxide ( $p\text{CO}_2$ ) in pigs are  $40 \pm 3$  mmHg.<sup>11</sup> Our results indicated that unthrifty piglets exhibited  $p\text{CO}_2$  within the normal range (41.2 mmHG). In this study, healthy piglets exhibited slightly higher than normal  $p\text{CO}_2$  (45.8 mm Hg), while unthrifty piglets had lower values ( $P = 0.05$ ), although in this case they were actually within the normal range. This finding is a bit surprising, as unthrifty piglets would be expected to be at least slightly stressed, and stress has been found to lead to reduced  $p\text{CO}_2$ . For example, micropigs that exhibited avoidance behaviors after exposure to a stressful stimulus had a lower  $p\text{CO}_2$  ( $P < 0.05$ ) during times when the stimulus was present than when the stimulus was not present (values not given).<sup>18, 19</sup> It is possible in this study that our assay

values for pCO<sub>2</sub> were slightly elevated above those from the assay used by PharmaVet Inc.<sup>11</sup> and thus pCO<sub>2</sub> values in unthrifty piglets may have been slightly decreased compared to normal values (as should be exhibited by the healthy piglets in this study). If that is the case, it is likely that reduced pCO<sub>2</sub> in unthrifty piglets may be due to stress or anxiety caused by any number of factors, including disease, anorexia or aphagia.

Neither group of piglets exhibited significantly increased epinephrine or norepinephrine levels. Significant results from other blood parameters, such as sodium, glucose and hematocrit, may have indicated that some piglets may have been experiencing weakness, hyperpnea, dehydration or loss of appetite. However, the effects of the stimuli that caused these signs may not have been intense or specific enough to elicit the fight or flight response that would cause an increase in stress hormones.

Necropsy results revealed that there were no significant differences in the presence of pulmonary lesions between unthrifty and healthy piglets ( $P = 0.88$ ). Grossly observable pulmonary lesions that were not attributed to the euthanasia process ( $n=3$ ) (i.e. those that were observed as signs of disease in live animals) were observed in both unthrifty ( $n=2$ ) and healthy ( $n=1$ ) pig groups. These lesions may have been caused by injuries sustained from a rough interaction with another piglet or from a previous illness, and given the difference in numbers of those observed between the two groups, may have been the primary reason the unthrifty piglets became anorexic.

Based on the results of this study, it is possible that unthrifty piglets may be identified by hematological analysis at a time when they may not be showing outward signs of disease. Early detection of unthriftiness may therefore allow for intervention strategies or early culling by producers. In addition, by assessing blood parameters such as sodium, hemoglobin, hematocrit and glucose, it may be possible for researchers to identify and therefore study piglets before they would normally be diagnosed with signs of PFTS (or other wasting diseases), providing the opportunity to assess early causative factors in this economically important but poorly understood syndrome.

## Implications

- Assessment of blood glucose, hematocrit, hemoglobin and sodium may be useful in early detection or study of piglet wasting diseases
- Blood parameters suggest that unthrifty piglets may be experiencing anorexia and weakness.

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## Tables and Figures

Table 2-1. Comparison of blood metabolite values in unthrifty (n = 32) and healthy (n = 26) nursery pigs (5.6 ± 1.3 kg). Differences are considered to be statistically significant at  $P \leq 0.05$ .

<b>Metabolite</b>	<b>Unthrifty</b>	<b>Healthy</b>	<b><i>P</i> Value Health</b>
Lactate (mmol/L)	3.5 ± 0.28	3.8 ± 0.32	0.47
Glucose (mg/dL)	78 ± 2.82	95 ± 3.12	0.0002
Hemoglobin (g/dL)	11.7 ± 0.29	9.9 ± 0.31	0.0002
Hematocrit (% PCV)	35 ± 0.85	29 ± 0.92	0.0002
iCa (mmol/L)	1.30 ± 0.02	1.36 ± 0.02	0.05
Sodium (mmol/L)	134 ± 0.66	137 ± 0.73	0.004
Potassium (mmol/L)	5.6 ± 0.14	5.4 ± 0.15	0.44
<i>p</i> CO <sub>2</sub> (mmHG)	41.2 ± 1.56	45.8 ± 1.73	0.05
<i>P</i> O <sub>2</sub> (mmHG)	49 ± 3.90	41 ± 4.33	0.17
pH	7.421 ± 0.01	7.409 ± 0.02	0.45
Cortisol (ng/ml)	22.260 ± 8.85	15.987 ± 9.08	0.34
Epinephrine (ng/ml)	0.14 ± 0.02	0.13 ± 0.02	0.86
Norepinephrine (ng/ml)	0.21 ± 0.01	0.22 ± 0.01	0.43

## CHAPTER THREE

### EVALUATION OF HYPOBARIC HYPOXIA EUTHANASIA FOR HEALTHY AND UNTHRIFTY NURSERY PIGS

#### ABSTRACT

Fifty-eight weaned nursery piglets ( $5.6 \pm 1.3$  kg) were utilized to compare electrophysiological, physiological and behavioral parameters of hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanasia. Piglets were euthanized by one of two methods: A) hypobaric hypoxia (HH) (approximate ascension of 36.9 m/sec) or B) carbon dioxide gas (CO<sub>2</sub>) (induction of approximately 20% of the chamber volume/minute) and were classified into two health groups: A) healthy or B) unthrifty. The euthanasia chamber was 1 m<sup>3</sup> in size. Health classification was determined by a certified veterinarian. Two piglets at a time were euthanized for each method by health status treatment (n=8, CO<sub>2</sub>-unthrifty; n=8, CO<sub>2</sub>-healthy; n=8, HH-unthrifty; n=5, HH-healthy). One animal in the pair was fitted with electrocardiogram (ECG) and electroencephalogram (EEG) monitoring devices, placed in the chamber and kept in the chamber until death was confirmed via ECG and EEG. In addition to ECG and EEG measurements, behavioral parameters were recorded. The average treatment times were HH,  $27.4 \pm 6.7$  minutes and CO<sub>2</sub>,  $13.8 \pm 5.1$  minutes. Time had a significant effect on EEG measurements in both CO<sub>2</sub> ( $P \leq 0.03$ ) and HH ( $P \leq 0.05$ ) treatments. There were no differences between HH and CO<sub>2</sub> in time to respiratory stress ( $P = 0.80$ ). Piglets euthanized via CO<sub>2</sub> reached a completely isoelectric state faster than piglets euthanized via HH ( $P = 0.009$ ). Behavioral data revealed gasping in 100% of CO<sub>2</sub> euthanized piglets during the first five minutes of treatment and only 28.6% of HH euthanized piglets during the same period. During HH, 57.1% of piglets fell down in the first five minutes while 76.9% of CO<sub>2</sub> piglets fell down during the

same time period. Further research should be conducted to investigate the impact of euthanasia method on electrophysiological, physiological and behavioral parameters.

**Key words: pig, euthanasia, behavior, hypobaric hypoxia**

## **INTRODUCTION**

Euthanasia is a necessary component of all swine management systems as it is sometimes the only option to alleviate pain and suffering of piglets that are born with congenital defects or are suffering from pre-weaning failure to thrive syndrome (PFTS). Piglets may also be euthanized if they are not expected to recover from a chronic illness or have suffered an injury such as a broken leg.

Although handler safety and training play a large role in selecting a euthanasia practice that is appropriate for on-farm use, piglet welfare is also a major consideration. An effective, repeatable, low stress method of on-farm piglet euthanasia is essential for maintaining industry-wide animal well-being standards. Commonly used methods of on-farm euthanasia for piglets include carbon dioxide gas asphyxiation and cervical dislocation (i.e. knocking). Current guidelines set forth by the American Veterinary Medical Association (AVMA) state that barbiturates, CO<sub>2</sub>, and penetrating captive bolt are acceptable methods of euthanasia for swine. Additionally, cervical dislocation is acceptable for piglets under three weeks of age.

While these methods are all approved and can be performed effectively, they have some accompanying disadvantages. Euthanasia methods that are visually distressing, such as knocking, are becoming increasingly scrutinized by the public as consumer and industry-wide concern for animal welfare grows. Handlers need to be properly trained to carry out euthanasia effectively so



that piglets do not experience any unnecessary stress from the process. Additionally, performing on-farm euthanasia can be stressful to the farm employee who performs the procedure. In a survey of swine farm employees who had performed knocking, conducted by Morrow *et al.* (2010), it was determined that 46.4% of respondents wished they would never have to euthanize piglets again in this manner. This result emphasizes the need for a euthanasia method that reduces the psychological stress of the employee.

While carbon dioxide is an effective, repeatable, and efficient method of euthanasia, it has also been reported that inhalation of carbon dioxide can cause adverse side effects such as breathlessness, convulsions, aversion, gasping, escape attempts and coughing in pigs (Mullenax and Dougherty 1963; Raj and Gregory 1995, 1996; Raj 1999; Dalmau *et al.* 2010). Additionally, if farm employees have not been properly trained to perform carbon dioxide euthanasia, unnecessary stress to the piglet can occur due to inadequate flow of CO<sub>2</sub> or poor chamber maintenance.

The preferential use of other gas mixtures such as argon for stunning (rendering the animal insensible) or euthanasia has been evaluated with some favorable results. Raj and Gregory (1995) subjected pigs to argon gas during feeding and saw no aversive side effects such as convulsing or gasping. However, due to argon's lack of odor and taste, this method could potentially be harmful to farm employees; therefore its use is not as prevalent within industry as carbon dioxide.

Due to concerns for piglet welfare and the emotional strain to swine farm employees associated with piglet euthanasia (Morrow *et al.*, 2010), alternative methods of low stress on-farm euthanasia are being explored. One potential euthanasia alternative is hypobaric hypoxia, a

method which produces hypoxic effects similar to those observed at high altitudes without an additional oxygen supply. Ascent to high altitude and the associated hypoxia can elicit such effects as sensory dullness, lassitude, neuromuscular weakness and a loss of consciousness caused by insufficient oxygen flow to the brain (Booth 1978). Furthermore, it has been proposed that hypoxia may be so acute that the associated loss of consciousness occurs without warning; mental disorientation, lack of coordination and drowsiness have also been observed (Busby *et al.*, 1976). Researchers have utilized dogs as models for hypobaric hypoxia since the early 1950's in order to examine the effects of high-altitude, low-oxygen atmospheres on astronauts and pilots (Cole *et al.*, 1953). Additionally, piglets have been used in high altitude simulations in order to examine the effects of high altitude exposure on internal organs for human medical applications (Schoene and Goldberg 1992; Stepanek *et al.*, 1998). However, hypobaric hypoxia has not previously been examined as a practical on-farm euthanasia method for piglets.

Humane euthanasia in a hypobaric chamber may allow swine producers to euthanize animals on-farm in a manner that could potentially be less stressful to the animal, more acceptable to the public, and less stressful for workers. Vizzier-Thaxton *et al.*, (2010) euthanized chickens via hypobaric hypoxia and determined it is humane method of stunning and eliminates the need for shackling chickens. Additionally, gas mixtures are not used in hypobaric hypoxia therefore eliminating the danger associated with handling gas cylinders. . If executed properly, hypobaric hypoxia euthanasia will enable producers and employees to euthanize more than one piglet simultaneously in a manner which is less stressful for the animals and is safer to the employee.

This objective of this experiment was to examine the electrophysiological , physiological and behavioral and electrophysiological responses to hypobaric hypoxia euthanasia in both healthy and unthrifty nursery pigs.

## **MATERIALS AND METHODS**

The Colorado State University and Kansas State University Institutional Animal Care and Use Committees approved protocols used in this experiment.

### ***Animals and experimental design***

Fifty eight piglets (22 barrows and 36 gilts;  $5.6 \pm 1.3$  kg; Danbred  $\times$  PIC 327, Hendersonville, TN, USA) from a commercial swine facility near Manhattan, KS were utilized in this experiment. The study took place over three days in October 2010. Piglets were approximately 21 to 28 days old and were housed on plastic slatted floors in 2.74 m x 3.05 m pens. Each pen contained 1 - cup waterer and 1 - double sided feeder. Piglets were fed a corn, grain sorghum and soybean meal diet ad libitum. Piglets were classified (by a certified veterinarian) into two health categories: healthy or unthrifty. Unthrifty piglets were free from abscesses or injury and were characterized through visible indicators of sickness, including but not limited to: coughing, apparent weakness, distended abdomen, apparent dehydration or malnourishment, lethargy and emaciation. Healthy piglets were those that did not exhibit any of the previous signs within the same groups. After being categorized by health status, piglets were weighed, ear tagged and marked with a livestock grease marker along the back.

Piglets were randomly assigned to one of two treatments: hypobaric hypoxia (HH) or carbon dioxide gas euthanasia (CO<sub>2</sub>). Piglets were euthanized in a chamber that was 1 m<sup>3</sup> in size with solid sides and was equipped with a plexiglass lid for behavioral observation. In the HH treatment, piglets were euthanized at an ascension rate of approximately 36.9 m/s. Piglets were

taken to a simulated peak altitude of 18000 meters and held at that altitude for a minimum of two minutes. The vacuum pump and ascension rate were monitored and controlled via an external laptop (Dell #PPO6S, Round Rock, TX, USA) operated by an engineer. The operator of the vacuum and CO<sub>2</sub> influx was the same for all treatments and had been properly trained to use the equipment associated with the euthanasia methods. Euthanasia via CO<sub>2</sub> took place by gas induction at 20% the volume of the chamber per minute for the duration of the treatment.

Within the chamber, there was a video camera (#PC131WR, Supercircuits, Austin, TX, USA; DVR #DVQ19N, Supercircuits, Austin, TX, USA) and a sling similar to the one developed by Panepinto (1983) that would accommodate one piglet. The sling was made from canvas and was suspended at the top of the chamber using two wooden dowels. The chamber accommodated two piglets, one in the sling and one moving freely on the floor of the chamber.

### ***Electroencephalogram preparation and recording***

Electroencephalograms (EEGs) were recorded on one piglet per pair prior to and during euthanasia. Prior to euthanasia, one pig was placed in the sling and electrodes (12mm x 28ga, Chalgren Enterprise, Inc., Gilroy, CA) were placed transcutaneously in a 5 channel montage (F3, F4, Cz, P3, P4; odd number = left hemisphere; even number = right hemisphere-) to record data using a diagnostic recording and analysis system (Sandman Spyder, Tyco Healthcare, Puritan Bennett Ltd., Kanata, ON, Canada). To assure good electrical contact with the electrodes, the scalp was defatted by rubbing vigorously with ethyl alcohol. Electrodes were placed over the frontal (F) and parietal (P) regions as well as along the median sagittal line (Cz) (Rose *et al.*, 1972). Approximate placement of EEG leads can be seen in Figure 1. The acquisition parameters to record EEG activity were set as follows: sensitivity = 5  $\mu$ V/mm; time constant = 0.3 s; high

filter (Hf) = 70 Hz; notch filter inserted; reference: on the bridge of the nose; ground: caudally to the external occipital protuberance; electrode impedance < 3 K $\Omega$ ; sampling rate 256 Hz. Seven EEG needles were used as active, reference, and ground electrodes. No local infiltration of lidocaine was performed around electrode placement sites. Electrocardiogram and respiratory rates were recorded via polygraphic channels (EKG: sensitivity = 70  $\mu$ V/mm, time constant = 0.1 s, Hf = 30 Hz; respiration - CHEST - : sensitivity = 20  $\mu$ V/mm, time constant = 0.3 s, Hf = 30 Hz) connected to alligator clips (thin cable for bridge electrode, Bionen S.a.S., Italy) and to a respiratory effort system (Sleepmate, MVAP, Newbury Park, CA).

EEG recording started when electrode placement was completed. The lid of the chamber was left open and the chamber left inactive for approximately 5 min after recording commenced in order to obtain sufficient EEG baseline values for comparison to treatment values. At the conclusion of the baseline observation period, a second pig was placed on the floor of the chamber, the lid was closed and the euthanasia treatment began.

The total EEG recording time was 30 min, including calibration and the initial impedance check. EEG data were stored in the acquisition station for later analysis.

### ***Electroencephalogram analysis***

Electroencephalogram data was analyzed and summarized by an electrophysiologist. Visual and quantitative EEG analyses were performed without knowing the identity of treatment groups. Before performing the quantitative analysis, all EEGs were examined visually to evaluate the background activity and artifacts. Special emphasis was given to artifact detection and elimination, because they strongly affect the frequency analysis of the EEG. Ocular

movements, cardiovascular and muscular activity, physiological rhythmic movements, or recording environment artifacts were noted and manually rejected.

Bio-electrical activity was analyzed using an integrated software program using the Fast Fourier Transform (FFT) algorithm (Persyst EEG Suite, Magic Marker, Persyst Development Corporation, Prescott, AZ < USA). EEG time points for the HH treatment included: Baseline (T1); after 10668 meters (T2); between 12192 and 13716 meters (T3); between 16764 and 18288 meters (T4) and lastly, after the piglet had been exposed to an equivalent elevation of 10668 meters for 9-10 minutes (T5). For the CO<sub>2</sub> treatment, five measurements were recorded for EEG power and frequency data. The first measurement, T1, refers to baseline measurement; T2 refers to 20 seconds post CO<sub>2</sub> induction, T3 refers to 300 seconds post CO<sub>2</sub> induction, T4 refers to 420 seconds post CO<sub>2</sub> induction and the final measurement, T5, refers to 540 seconds post CO<sub>2</sub> induction.

In the HH treatment for all the piglets, at least 18 replications of 2-s artifact-free epochs were selected through baseline (T1) whereas at least 6 replications at the other time points (T2, T3, T4 and T5). In the CO<sub>2</sub> treatment for all the piglets, at least 30 replications of 2-s artifact-free epochs were visually selected through baseline (T1) whereas and at least 12 replications at the other time points (T2, T3, T4 and T5). Fewer replications were available for selection in HH treatment piglet data, due to artifacts during recording. The baseline period was longer than the other timepoints and therefore more data was selected. Fast Fourier Transform was calculated for each channel and averaged. The spectral bands of delta (0.5 - 4.0 Hz), theta (4.1 - 8.0 Hz), alpha (8.1 - 12.0 Hz), and beta (12.1 - 30.0 Hz) waves were calculated and expressed as relative power (%; alpha, beta, delta and theta waves) and median frequency (Hz). Total power ( $\mu V^2$ ), of the

entire spectrum (0.5 - 30.0 Hz) was also calculated. A visual representation of wave patterns for alpha, beta, theta and delta can be seen in Figure 2.

Relative power is a ratio between a given band's absolute power and the sum of the absolute powers of the entire frequency band spectrum, multiplied 100 times. To standardize absolute power across our animal population, relative power as well as median, mean, and peak frequency were used in the statistical analyses.

The peak frequency refers to the peak value in a selected band of the frequency spectrum. The mean frequency refers to the arithmetical mean value in a selected band of the frequency spectrum. The median frequency refers to that frequency value in a selected band of the frequency spectrum that divides the selected band of the frequency spectrum in two part of equal absolute power.

Electroencephalogram waves were also evaluated in order to determine the amount of time each treatment took to elicit an isoelectric response in the piglet. For pigs euthanized via HH, time was measured from the point at which 7010 m was reached to when an isoelectric EEG was seen accompanied by ECG artifacts. When evaluating data from CO<sub>2</sub> euthanized pigs, time was measured from the start of treatment to the emergence of an isoelectric EEG accompanied with ECG artifacts and then from that point to a totally isoelectric EEG. As with HH pigs, time from the point at which artifacts were seen in the ECG to a totally isoelectric EEG was also measured. During euthanasia for several pigs, the EEG electrodes became dislodged from the piglet due to excessive movement of the piglet.

### ***Electrocardiogram analysis***

Heart rate data was collected from ECG recordings collected during baseline and euthanasia.

Heart rate (bpm) for HH euthanized pigs were as followed: baseline = prior to start of euthanasia;

initial = at peak altitude; one min = one min at peak altitude; two min = two min at peak altitude). Heart rate (bpm) for CO<sub>2</sub> euthanized pigs over time during treatment (baseline = prior to start of euthanasia; initial = at gas induction; one min = one min after induction; two min = two min after induction). All electrocardiogram values were counted manually from observations of ECG wave data using the same output and software as electroencephalogram data..

### ***Behavioral analysis***

During euthanasia, animal behaviors were recorded using two video cameras. Piglets euthanized in the Panepinto-like sling were recorded using a hand-held video recorder (Sanyo Xacti HD, Sanyo, San Diego, CA, USA) mounted on a tripod and piglets euthanized on the floor of the chamber were videoed with video cameras that had been pre-mounted within the euthanasia chamber (#PC131WR, Supercircuits, Austin, TX, USA). One observer watched the video recordings for both the HH and CO<sub>2</sub> euthanized piglets. Twenty-five minutes of video recording was reviewed and analyzed for each piglet. Piglets on the floor were monitored for the numbers of occurrences of paddling, gasping, convulsing and falling down. Piglets restrained in the sling were monitored for the number of occurrences of gasping, struggling, convulsing and vocalizations. Descriptions of behaviors are shown in Table 1. Vocalizations were not recorded from pigs on the floor due to the lack of audio capabilities of the camera inside the chamber. The results of behavioral measurements are shown in Table 2 are the percentage of piglets that exhibited each measured behavior during each time segment.

Death of the piglet in the sling was confirmed via EEG and ECG by an electrophysiologist. Death of the piglet on the floor was determined by visual observation of a cessation of movement and respiration for five consecutive minutes. Upon confirmation of



death, the chamber was returned to ambient environmental conditions and piglets were removed from the chamber.

### ***Statistical analyses***

Statistical analyses were conducted using SAS 8.2 (SAS Institute Inc., Cary, North Carolina, USA). Electroencephalogram data (time to isoelectric signal) was analyzed using PROC MIXED with the fixed effects of euthanasia method, health status and time. Data for 21 pigs was included for time to an isoelectric EEG (CO<sub>2</sub> healthy – 6; CO<sub>2</sub> unthrifty – 8; HH healthy – 3; HH unthrifty – 4). Electroencephalogram data for total power, relative power and median frequency were analyzed on an individual pig basis using mixed model procedures appropriate for a randomized block design with repeated measures as described by Littell et al. (1998) and SAS (2001). Pig (health) was included in the model as random effect. The subject effect of the repeated statement was the pig\*health\*time interaction. First order autoregressive (AR1) covariance structure was used. All variables in the models were considered as class variables. EEG data for 15 pigs is included in this analysis (CO<sub>2</sub> healthy – 5; CO<sub>2</sub> unthrifty – 4; HH healthy – 2; HH unthrifty – 4).

## **RESULTS**

### ***Electroencephalogram data***

Results of EEG data, in terms of total treatment time observed until the appearance of an isoelectric EEG, demonstrated that time to onset of respiratory distress was not different between HH (8.9 ± 3.8 min) and CO<sub>2</sub> (5.1 ± .9 min) euthanized piglets ( $P = 0.80$ ) or healthy piglets (7.4 ± 3.0 min) and unthrifty piglets (6.0 ± 3.1 min;  $P = 0.42$ ). Additionally, there were no significant differences in the amount of time elapsed from the onset of respiratory distress to the point of an

isoelectric EEG accompanied by an ECG signal ( $P = 0.14$ ). However, when evaluating the length of time elapsed from the appearance of an isoelectric EEG with an ECG signal to the point of a completely isoelectric EEG signal (with no ECG artifacts), there was a significant ( $P = 0.009$ ) treatment effect; piglets euthanized via CO<sub>2</sub> reached the point of a completely isoelectric state faster than piglets euthanized via hypobaric hypoxia. The average treatment time for HH and CO<sub>2</sub> were  $27.4 \pm 6.7$  minutes and  $13.8 \pm 5.1$ , respectively. These results indicate that euthanasia via CO<sub>2</sub> was quicker than HH.

Electroencephalogram power measurement results indicated that time had a significant effect on all measured variables for CO<sub>2</sub> ( $P \leq 0.03$ ) piglets and for HH piglets in the relative power alpha, beta and delta bands and also in median frequency and total power ( $P \leq 0.05$ ). There was a health status  $\times$  time interaction on median frequency and relative power alpha, beta and delta in HH pigs ( $P \leq 0.02$ ) but no significant interactions and no health effect in CO<sub>2</sub> piglets ( $P \geq 0.05$ ).

Graphs for all measured variables can be seen in Figures 3-8. Within the HH treatment, T3 and T4 were significantly lower than T5 in the relative power alpha and beta (Figures 3 and 4, respectively) bands ( $P \leq 0.002$ ) and T1 was significantly higher than T4 in the alpha band ( $P = 0.02$ ) but significantly lower in the delta (Figure 5) band ( $P = 0.02$ ). Also in relative power beta, T2 was significantly lower than T5 ( $P = 0.0001$ ) and T1 was significantly higher than T2 and T3 ( $P = 0.02$ ). In relative power delta, T4 was significantly higher than T5 ( $P = 0.0003$ ). There were no significant differences present in the relative power theta band (Figure 6). When evaluating median frequency (Figure 7), T3 was significantly lower than T5 ( $P = 0.02$ ). In the total power measurements (Figure 8), T1 was significantly lower than T2 ( $P = 0.04$ ).

Within the CO<sub>2</sub> treatment, T2 was significantly lower than T4 and T5 in relative power alpha and beta ( $P \leq 0.02$ ), (Figures 3 and 4, respectively). Additionally, T3 was significantly lower than T5 in alpha and beta ( $P \leq 0.02$ ) and in the alpha band T3 was also significantly lower than T4 ( $P \leq 0.05$ ). When evaluating the relative power delta band (Figure 5), T2 and T3 were significantly higher than T5 ( $P \leq 0.03$ ). The relative power theta band (Figure 6), showed that T1 was significantly higher than all subsequent time points ( $P = 0.001$ ). Total power measurements (Figure 8), showed that T2 was significantly greater than all subsequent time points ( $P \leq 0.05$ ). Additionally, median frequency (Figure 7), at T2 and T3 were significantly lower from T5 ( $P = 0.02$ ).

### ***Behavioral Data***

The percentage of piglets that exhibited observed behaviors in both HH and CO<sub>2</sub> euthanized piglets can be seen in Table 2. Behavior was not compared statistically between treatments however, several numerical differences can be ascertained from the data when comparing the two euthanasia treatments.

All piglets (100%) euthanized via CO<sub>2</sub>, both on the floor and in the sling, exhibited gasping behavior in the first five minutes of treatment. Additionally, 100% of piglets euthanized on the floor gasped in the 5-10 minute time period whereas only 60% of piglets euthanized in the sling gasped during the same time period. When evaluating paddling behavior, 92.3% of CO<sub>2</sub> piglets on the floor exhibited paddling in the first five minutes of treatment, compared to only 42.9% of HH piglets on the floor. However, only 15.4% of CO<sub>2</sub> piglets on the floor exhibited paddling during the 5-10 period while 57.1% of HH piglets on the floor paddled during the same time period.

When examining convulsions, 38.5% of CO<sub>2</sub> piglets on the floor and 60% in the sling convulsed in the first 5 minutes of treatment whereas 0% of HH piglets on the floor and 60% in the sling convulsed during the same time period. Moving on the the 5-10 minute time period, 71.4% of HH pigs on the floor and 80% in the sling exhibited convulsing behavior compared to only 69.2% of floor and 0% of sling CO<sub>2</sub> piglets.

During the five minute time period, 76.9% of CO<sub>2</sub> piglets on the floor fell down during treatment compared to only 57.1% of floor euthanized HH piglets. Conversely, 85.7% of piglets on the floor in the HH treatment fell during the 5-10 minute time period compared to 0% of CO<sub>2</sub> floor piglets.

## **DISCUSSION**

The results of this experiment indicate that although both methods of euthanasia are effective, they differ in the effects they have on the piglet. Hypobaric hypoxia occurs when the atmospheric partial pressure of oxygen is less than the physiological level necessary to properly oxygenate body tissues (Booth, 1978). Oxygen sensors in the brain stem detect low levels of oxygen and initiate an increase in heart and respiration rate. Consequently, the amount of oxygen to the brain should increase, however, the lack of oxygen in the environment prevents proper oxygenation of brain tissue and eventually animals are rendered unconscious before death. Piglets euthanized via HH may have experienced headache, sensory dullness, dyspnea, dizziness and loss of consciousness (Booth, 1978). Furthermore, Booth (1978) affirmed that hypoxia is not to be confused with suffocation or asphyxiation, such as CO<sub>2</sub> euthanasia, and that suffocation is not a factor in ascent to high altitudes or during decompression. The length of exposure to low oxygen levels is a determinant of the intensity of the previously mentioned side effects of HH. As the duration of exposure increases, so does the intensity of the side effects.

Over time, in both treatment groups, the frequency of all waves declined to a low frequency, which is indicative of vegetative state, coma or loss of consciousness (Baars et al. 2003). This pattern can also be seen in both groups in the total power and median frequency variables. However, in the relative power alpha and beta bands, (Figures 3 and 4,) there is an increase in power towards the end of treatment. It's important to note that these measurements can be attributed to ECG artifacts; meaning that although the EEG was isoelectric, an ECG signal was still present and may have caused interference. Artifacts do not appear in the delta and theta bands because those frequency bands exist in ranges that are too low to register ECG artifacts. Gerritzen et al. (2006) affirms that the suppression of alpha and beta waves with the occurrence of theta and delta waves are seen during unconsciousness while the suppression of theta and delta waves will lead to an irreversible isoelectric EEG signal. This agrees with the present results in which theta and delta waves were at higher frequencies at T4 ( $\text{CO}_2 = 420$  seconds;  $\text{HH} = 360$  seconds) than alpha and beta, indicating unconsciousness before an isoelectric EEG.

Piglets euthanized via  $\text{CO}_2$  exhibited an almost immediate decrease in relative power alpha, beta and theta (Figures 3, 4 and 6, respectively). Piglets euthanized via HH also exhibited decreased brain activity in the alpha, beta and theta bands, however it appears to be less immediate. This is indicative of depressed brain activity and is confirmed by Gerritzen et al. (2008) who saw a decline to lower EEG frequencies in piglets after approximately  $19 \pm 1.1$  seconds of submersion in a 70%  $\text{CO}_2 + 30\%$   $\text{O}_2$  environment. Furthermore, minimal brain activity was observed after submersion in the gas mixture for greater than  $33 \pm 2$  seconds. Although the present results do not show piglets reaching an isoelectric state at approximately 33 seconds, it's important to note that the euthanasia chamber was not prefilled with  $\text{CO}_2$  prior to

the onset of treatment. However, it is not clear from Gerritzen et al. (2008) whether or not the test box was prefilled so it is difficult to assess the speed of loss of consciousness in comparison to this study. There was a slight increase of relative power in the delta bands in both HH and CO<sub>2</sub> piglets after the onset of treatment. This may be attributed to a sudden awakened state that could have been caused by the noise of the vacuum chamber or the initial inhalation of CO<sub>2</sub>.

Relative power delta and total power in both groups of piglets revealed an increase in activity before a gradual decrease to an isoelectric state. These results disagree with that of Goel et al. (1996) which found an increase in power in the medium and high frequency bands (theta, alpha and beta, respectively) during hypoxic stress in one week old piglets, whereas the piglets in this study did not show an increase in the high frequency bands. This differentiation could possibly mean that the procedures in this study caused less hypoxic stress than that of Goel et al (1996). Total power ( $\mu V^2$ ) also increased at the onset of treatment before gradually decreasing throughout the treatment in both HH and CO<sub>2</sub> euthanized piglets.

Coenen et al. (2005) found through EEG, ECG and behavioral analysis that in an oxygen containing condition, chickens reach unconsciousness slower and die later than in an oxygen lacking setting such as a mixture of CO<sub>2</sub> and N<sub>2</sub>. Additionally, Coenen et al. (2005) found that although the speed of euthanasia was much faster in an oxygen free environment, such conditions led to strong signs of distress and agitation. These results, paired with results from the current study may indicate that even though CO<sub>2</sub> is aversive initially it is a faster method. Additionally, behavior responses are similar between the two methods however the behaviors occur on a different time scale.

Electrocardiograph results indicated that piglets in the HH treatment experienced an increase in heart rate after the onset of treatment. After two minutes, heart rate had decreased to below baseline levels. These results agree with Hainsworth et al. (2007) and Vogel and Harris (1967) who affirmed that hypobaric hypoxia results in an increase in resting heart rate during altitude exposure and during simulated exposure utilizing a hypobaric chamber. Piglets euthanized via CO<sub>2</sub> also demonstrated a decline in heart rate after several minutes of treatment, however unlike HH piglets, heart rate did not increase immediately as in the HH treatment. Additionally, although a statistical comparison was not made between treatment methods, heart rate appears to have declined more quickly in CO<sub>2</sub> piglets than in HH piglets.

Death via excessive CO<sub>2</sub> inhalation is similar to death from HH however, the side effects of CO<sub>2</sub> appear to be more harsh than those of HH. Carbon dioxide has been shown to cause aversive behaviors in pigs such as convulsing, escape attempts, head shaking and gasping (Mullenax and Dougherty 1963; Troeger and Woltersdorf 1991; Raj and Gregory 1995). Deiss et al (2006) reported that 8 out of 10 piglets that were exposed to a CO<sub>2</sub> concentration of 80%, exhibited violent jumps, respiratory difficulty and vocalizations. Raj (1999) found that 50% of pigs exposed to high concentrations of CO<sub>2</sub> (80-90%) showed reactions such as head shaking and convulsions during exposure and all pigs hyperventilated when exposed to CO<sub>2</sub>. The negative response of pigs to CO<sub>2</sub> inhalation reported in these studies suggests a need to find an equally effective, yet lower stress method of on-farm swine euthanasia.

Numerous studies have been conducted on the behaviors associated with exposing piglets to carbon dioxide (Raj and Gregory 1995, Velarde et al. 2007, Dalmau et al. 2010). In the present experiment, 100% of CO<sub>2</sub> piglets, both in the sling and on the floor, exhibited gasping during the first five minutes demonstrating similar results to Velarde et al (2007) both indicating

breathlessness with CO<sub>2</sub> exposure. Additionally, current data show that 80% of piglets euthanized in the sling via CO<sub>2</sub> struggled or attempted to escape from the irritant during the first five minutes and 76.9% of piglets euthanized via CO<sub>2</sub> on the floor exhibited convulsions during the first five minutes of euthanasia. These data agree with Raj and Gregory (1995) that inhalation of carbon dioxide can cause aversive behavior in a majority of piglets and may cause shortcomings in piglet welfare. The high percentage of piglets that exhibited these behaviors during the first five minutes of CO<sub>2</sub> treatment is important to note because CO<sub>2</sub> causes unconsciousness and death quickly as evidenced by the EEG data. This is evident when evaluating the percentage of piglets that exhibited stress induced behaviors during later stages of CO<sub>2</sub> treatment. As aforementioned, 76.9% of CO<sub>2</sub> piglets on the floor fell down in the first five minutes and when examining paddling behavior, 92.3% of CO<sub>2</sub> floor piglets paddled during the first five minutes, yet only 15.4% paddled during the 5-10 minute time segment. Additionally, 80% of CO<sub>2</sub> piglets in the sling convulsed during the first five minutes. Behaviors during the 10-15 minute time segment weren't exhibited because piglets were deceased by that stage of treatment however, it's important to note that this pattern was not the same in HH piglets. Similarly, 100% of piglets euthanized via HH on the floor of the chamber exhibited convulsions. It's important to note that convulsing does not signify seizing, as seizing could be identified through EEG wave data. This difference may be attributed to differences in ascension rate, although Breazile and Kitchell (1969) do not provide exact values for ascension rate or specific requirements for producing hypoxia without convulsions.

Hypobaria and hypoxia have long been studied in dogs and pigs by aeronautical institutions to determine the effects of oxygen deprivation on humans (Bancroft and Dunn 1965, Booth 1978, Stepanek et al. 1998). To the knowledge of the investigators, this experiment is the



first of its kind to fully euthanize piglets via hypobaric hypoxia. Previous research by Breazile and Kitchell (1969) stated that hypoxia can serve as an effective method of euthanasia that does not cause the animal to struggle but gives way to a state of natural sleep. This is in direct contrast to the present study's findings, as 100% of piglets in the sling struggled during the first five minutes. Lukatch *et al.* (1997) notes that in rats, an isoelectric EEG can be determined from the occurrence of 10% or less of baseline total power. Although EEG total power (Figure 8) results indicate that throughout the first five minutes total power decreased, the total power value at approximately five minutes was not 10% or less than baseline total power. This relationship indicates that piglets were not unconscious five minutes into treatment. Similarly, 71.4% of piglets euthanized via HH on the floor of the chamber exhibited convulsions during the 5-10 minute time segment. The absence of convulsions during the first five minutes but rather high presence during later stages may indicate that HH prolongs stress that is associated with euthanasia. It's important to note that convulsing does not signify seizing, as seizing could be identified through EEG wave data. This difference may be attributed to differences in ascension rate, although Breazile and Kitchell (1969) do not provide exact values for ascension rate or specific requirements for producing hypoxia without convulsions.

Stepanek *et al.* (1998) reported ataxia and lethargy in piglets that were exposed to HH and then recompressed. Data from this study also showed that 57.1% of piglets euthanized on the floor of the chamber via HH during the first five minutes were ataxic and fell down. This behavior occurred repeatedly and often during the treatment which is reflected through the 85.7% of HH piglets on the floor that fell down during the 5-10 minute time segment. During this time, HH piglets showed decreasing total power (Figure 8), and at T5 (540 seconds) total power was less than total power at T1 (baseline), however, as previously mentioned in regards to

Lukatch *et al.* (1997), total power at T5 was not 10% or less than baseline. This may indicate that HH does not quickly yield unconsciousness and can be detrimental to the piglet. Similarly, 76.9% of piglets euthanized via CO<sub>2</sub> fell down during the first five minutes however 0% of CO<sub>2</sub> piglets on the floor fell down during the 5-10 and 10-15 minute time segments, indicating that they fell down and became unable to stand after the first five minutes. Due to the physiological similarities between the two methods of euthanasia, it is not surprising that piglets from both treatments exhibited similar behaviors..

Behavioral data from this trial provides inconclusive evidence as to whether hypobaric hypoxia (HH) euthanasia is a more preferential and humane method of euthanasia than CO<sub>2</sub> euthanasia. While fewer HH euthanized piglets on the floor fell down and exhibited gasping, paddling, convulsing behaviors (57.1%, 28.6%, 42.9% and 0%, respectively) during the first five minutes of treatment than CO<sub>2</sub> piglets on the floor (76.9%, 100%, 92.3% and 38.5%, respectively), the percentage of HH piglets on the floor that exhibited these same behaviors was higher than CO<sub>2</sub> piglets on the floor during the 5-10 minute period. This occurrence may point to less stress for piglets during the early stages of HH, however the increase of piglets exhibiting stress induced behaviors during later stages of treatment indicates that although the stress isn't immediate, it may be prolonged. These results may present a dichotomy for producers who may be faced with choosing a less stressful yet prolonged euthanasia method compared to a quicker but possibly harsher treatment such as CO<sub>2</sub> euthanasia.

There is a need for more low stress euthanasia methods in the swine industry, in regards to employee emotional comfort level and humaneness of the method. Hypobaric hypoxia may meet this need however, more thorough research utilizing EEG and ECG equipment should be conducted to fully grasp the brain's activity during death. Additionally, collecting blood

samples throughout the treatment period and immediately after confirmation of death may aid in evaluating the stress associated with the varied methods of on-farm euthanasia. Further examining the rate of ascension in HH may uncover a more efficient rate in which to bring forth unconsciousness in piglets, which would ultimately be beneficial to the piglet's well-being. Additionally, proper maintenance of the euthanasia chamber is vital in efficiently carrying out both methods of euthanasia, however, at this time hypobaric hypoxia is not a viable method of on-farm euthanasia.

## IMPLICATIONS

While this research is the first to explore euthanasia via hypobaric hypoxia in piglets, there is still much to be revealed about whether or not hypobaric hypoxia is a more humane method of on-farm euthanasia. Although CO<sub>2</sub> euthanasia may be more aversive, it does serve as a faster form of euthanasia than HH. Further research should utilize blood sampling throughout the euthanasia to monitor the changes in blood hormones and catecholamines. Additionally, it would be valuable to determine the altitude at which HH euthanasia should be performed to be efficient and cause the least amount of stress.

## ACKNOWLEDGMENTS

The authors express their sincere gratitude to Hyatt Frobose, Jackie Hand, Scott Hill, Kyla Krissek, Megan Kulas, Melanie Peele, and Karen Sellins for their assistance with the preparation and completion of this trial. Additionally, many thanks are given to Kent and Marian Condray who permitted this trial to take place at their swine operation.

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## Tables and Figures

Table 3-1. Description of observed behaviors for piglets euthanized (HH and CO<sub>2</sub>) in the sling and on the floor of the chamber.

<b>Sling Behavior</b>	<b>Description</b>
Gasping	The visual observation of respiratory thoracic movement during labored breathing
Struggling/Escape	Voluntary movement of the legs or body in an attempt to free itself from the sling
Convulsing	Involuntary contraction of the muscles, producing contortion of the body, back or limbs
Vocalize	Vocalizations produced by the piglet
<b>Floor Behavior</b>	--
Gasping	The visual observation of respiratory thoracic movement during labored breathing
Paddling	Movement of the limbs in a running motion while the animal is lying on its side
Convulsing	Involuntary contraction of muscles, producing contortion of the body, back or limbs
Falling Down	Losing footing and falling completely down onto floor

Table 3-2. Percentage of piglets that exhibited observed behaviors, in five-minute segments, for the duration of euthanasia in both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) treatments (n = CO<sub>2</sub> floor – 13; CO<sub>2</sub> sling – 5; HH floor – 7; HH sling – 5) .

	<b>HH</b>				<b>CO<sub>2</sub></b>		
	<i>0-5 min</i>	<i>5-10 min</i>	<i>10-15 min</i>	<i>15-20 min</i>	<i>0-5 min</i>	<i>5-10 min</i>	<i>10-15 min</i>
<b>Floor</b>							
Gasp (%)	28.6	100	57.1	28.6	100.0	100.0	0.0
Paddle (%)	42.9	57.1	14.3	0.0	92.3	15.4	0.0
Convulse (%)	0.0	71.4	57.1	14.3	38.5	69.2	0.0
Fall (%)	57.1	85.7	0.0	0.0	76.9	0.0	0.0
<b>Sling</b>							
Gasp (%)	20.0	80.0	20.0	20.0	100.0	60.0	0.0
Struggle/Escape (%)	100.0	20.0	0.0	0.0	80.0	0.0	0.0
Convulse (%)	60.0	80.0	20.0	0.0	60.0	60.0	0.0
Vocalize (%)	60.0	0.0	0.0	0.0	100.0	0.0	0.0

Figure 3-1. Approximate placement of cranial electroencephalogram (EEG) electrodes during euthanasia in piglets  $5.6 \pm 1.3$  kg.

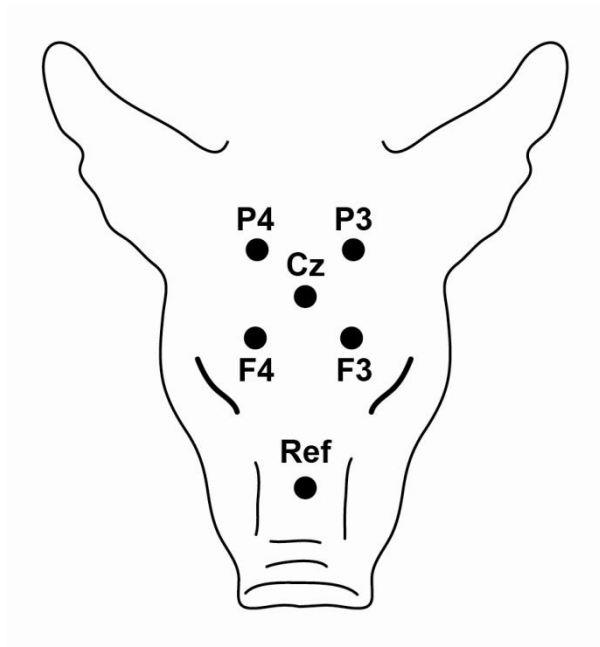


Figure 3-2. Illustration of normal electroencephalogram wave patterns for alpha (8.1 – 12.0 Hz), beta (12.1 – 30.0 Hz), theta (4.1 – 8.0 Hz) and delta (0.5 – 4.0 Hz) waves.

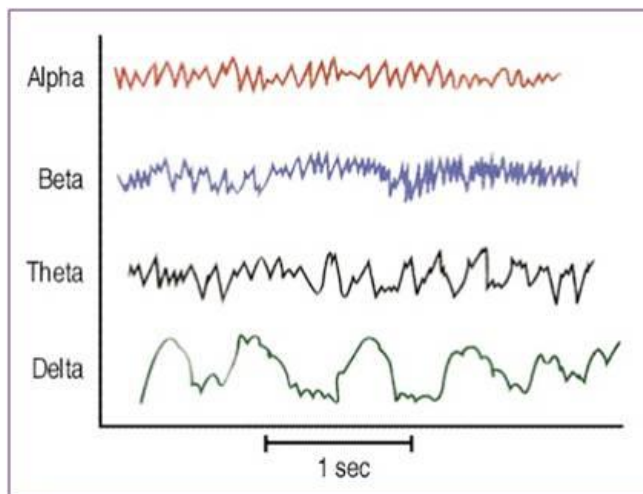




Figure 3-3. Electroencephalogram relative power (%) alpha values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Power measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.

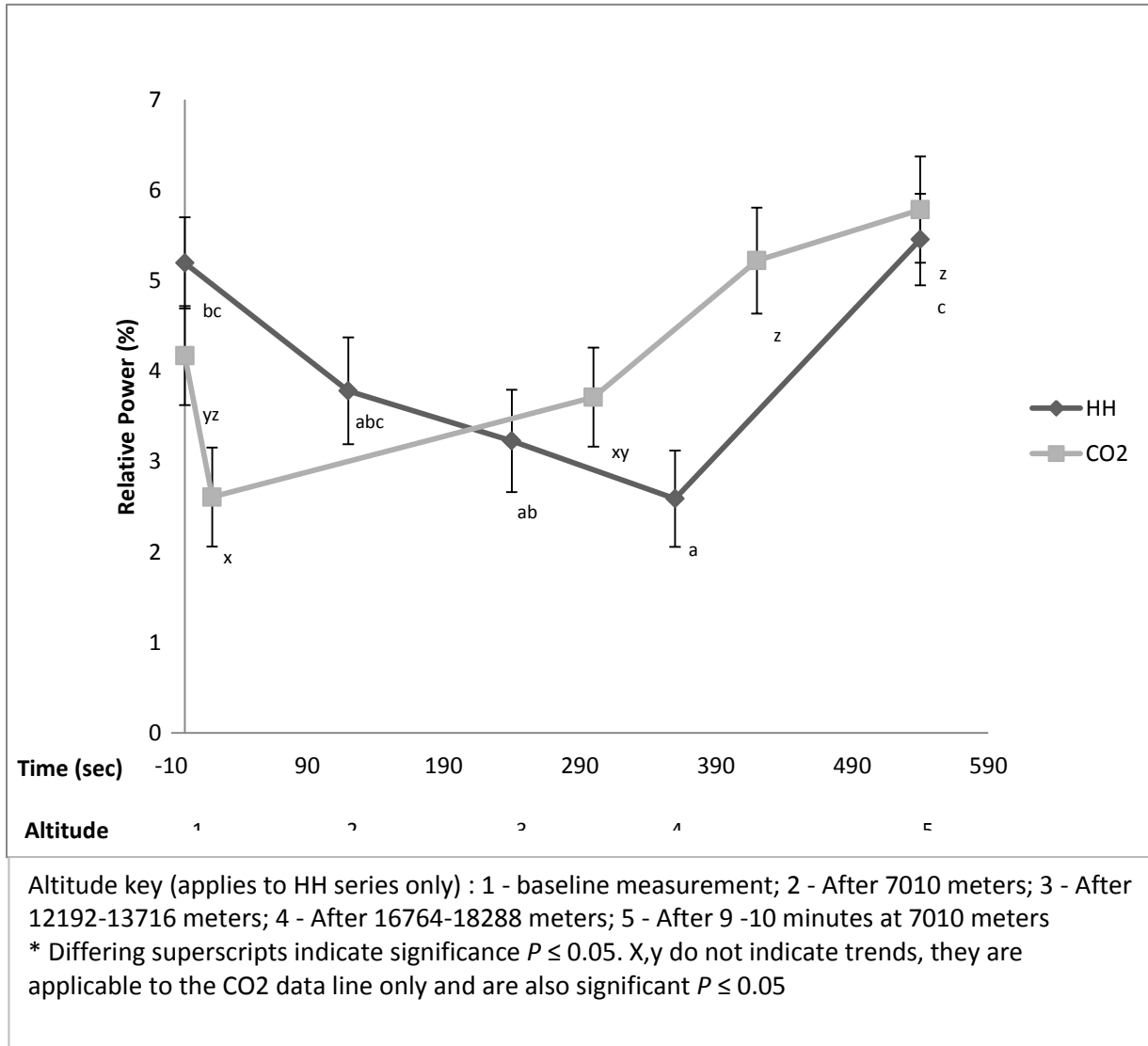


Figure 3-4. Electroencephalogram relative power (%) beta values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Power measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.

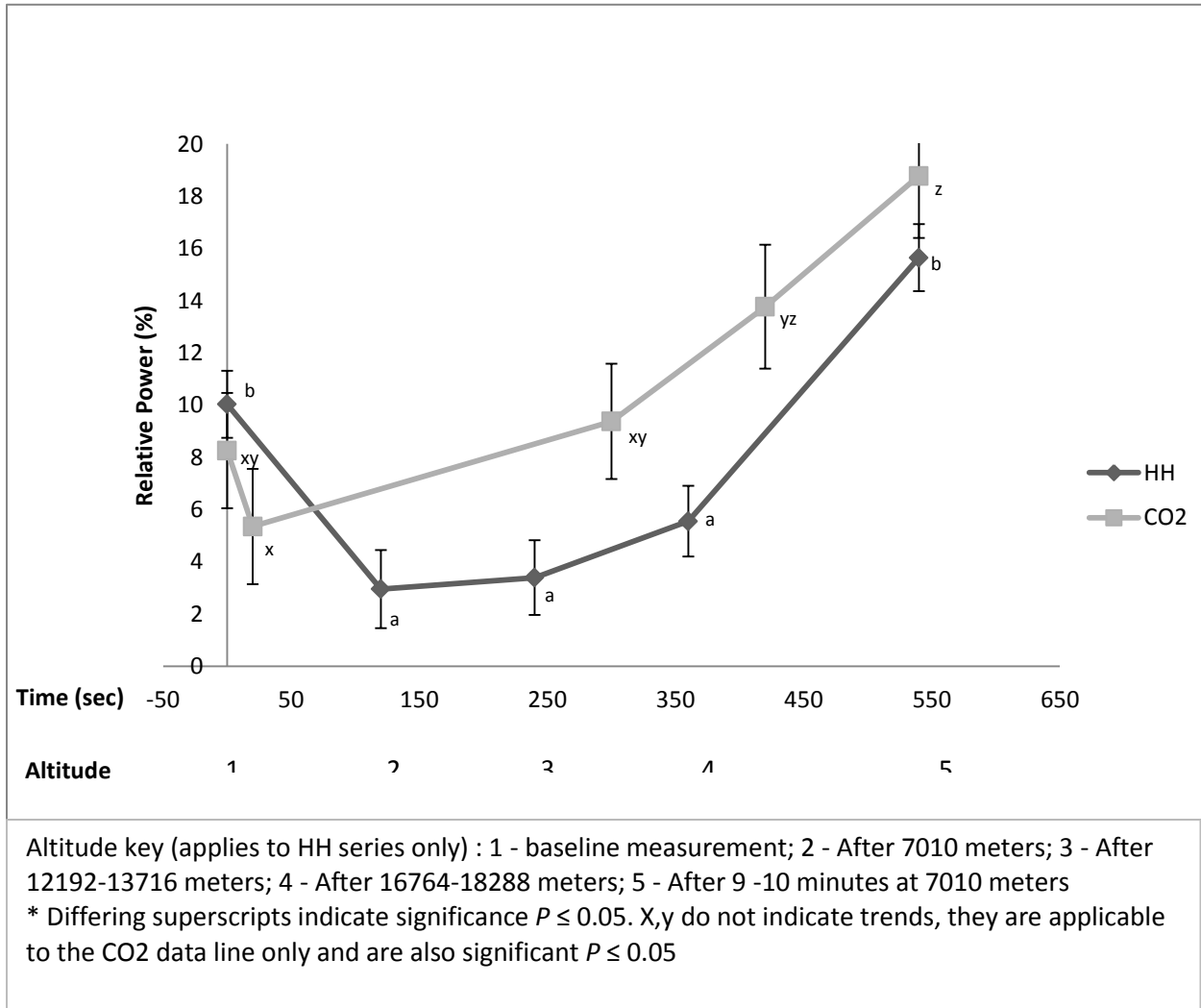


Figure 3-5. Electroencephalogram relative power (%) delta values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Power measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.

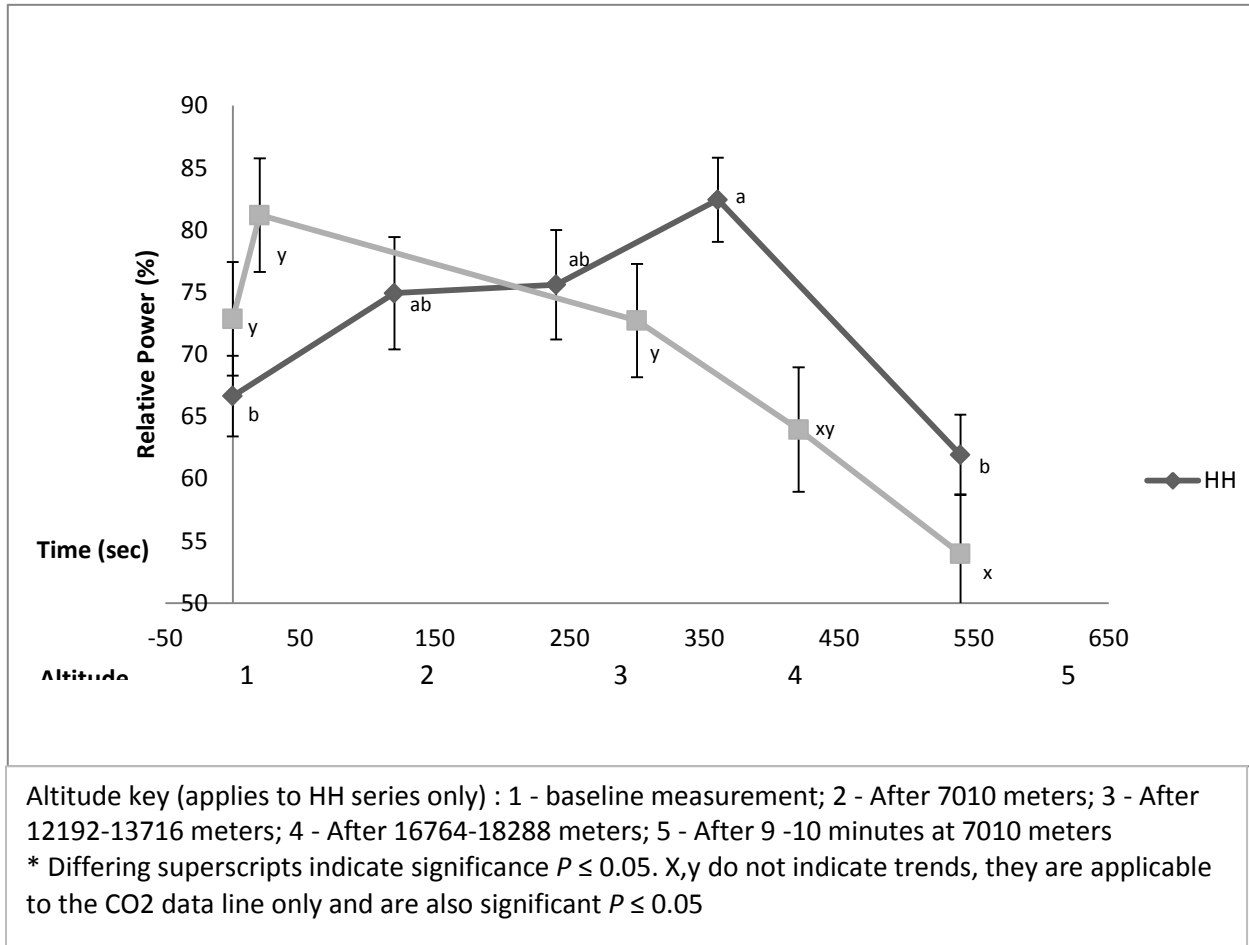
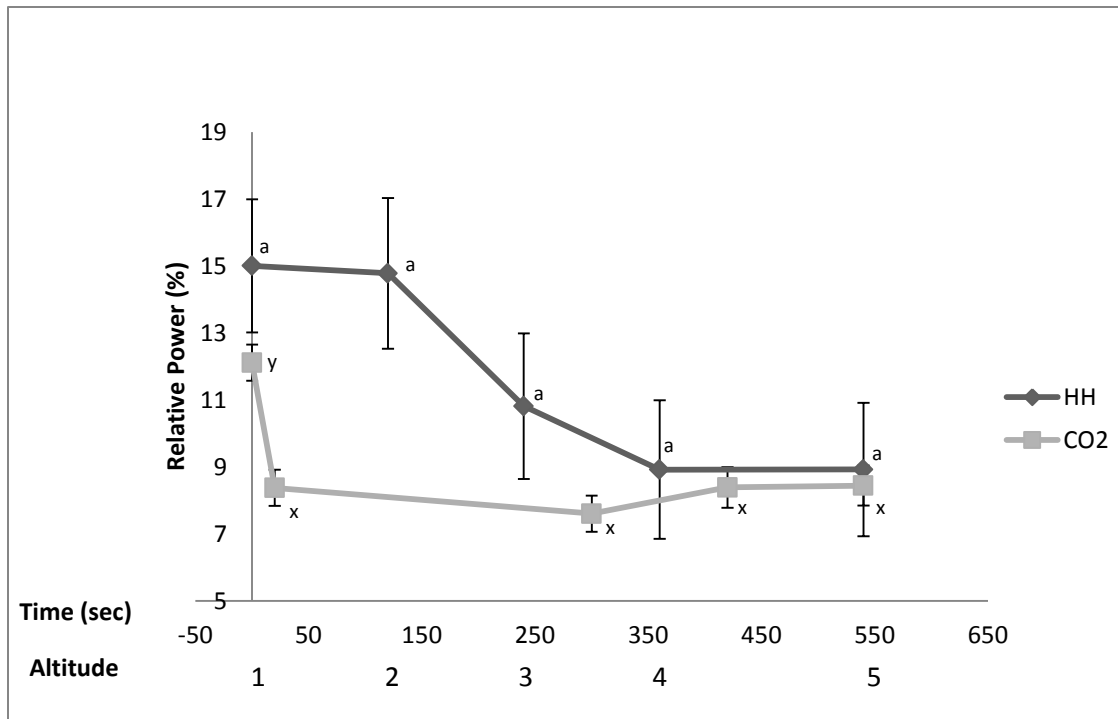


Figure 3-6. Electroencephalogram relative power (%) theta values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Power measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.



Altitude key (applies to HH series only) : 1 - baseline measurement; 2 - After 7010 meters; 3 - After 12192-13716 meters; 4 - After 16764-18288 meters; 5 - After 9 -10 minutes at 7010 meters  
 \* Differing superscripts indicate significance  $P \leq 0.05$ . X,y do not indicate trends, they are applicable to the CO<sub>2</sub> data line only and are also significant  $P \leq 0.05$

Figure 3-7. Electroencephalogram median frequency (Hz) values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Median frequency measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.

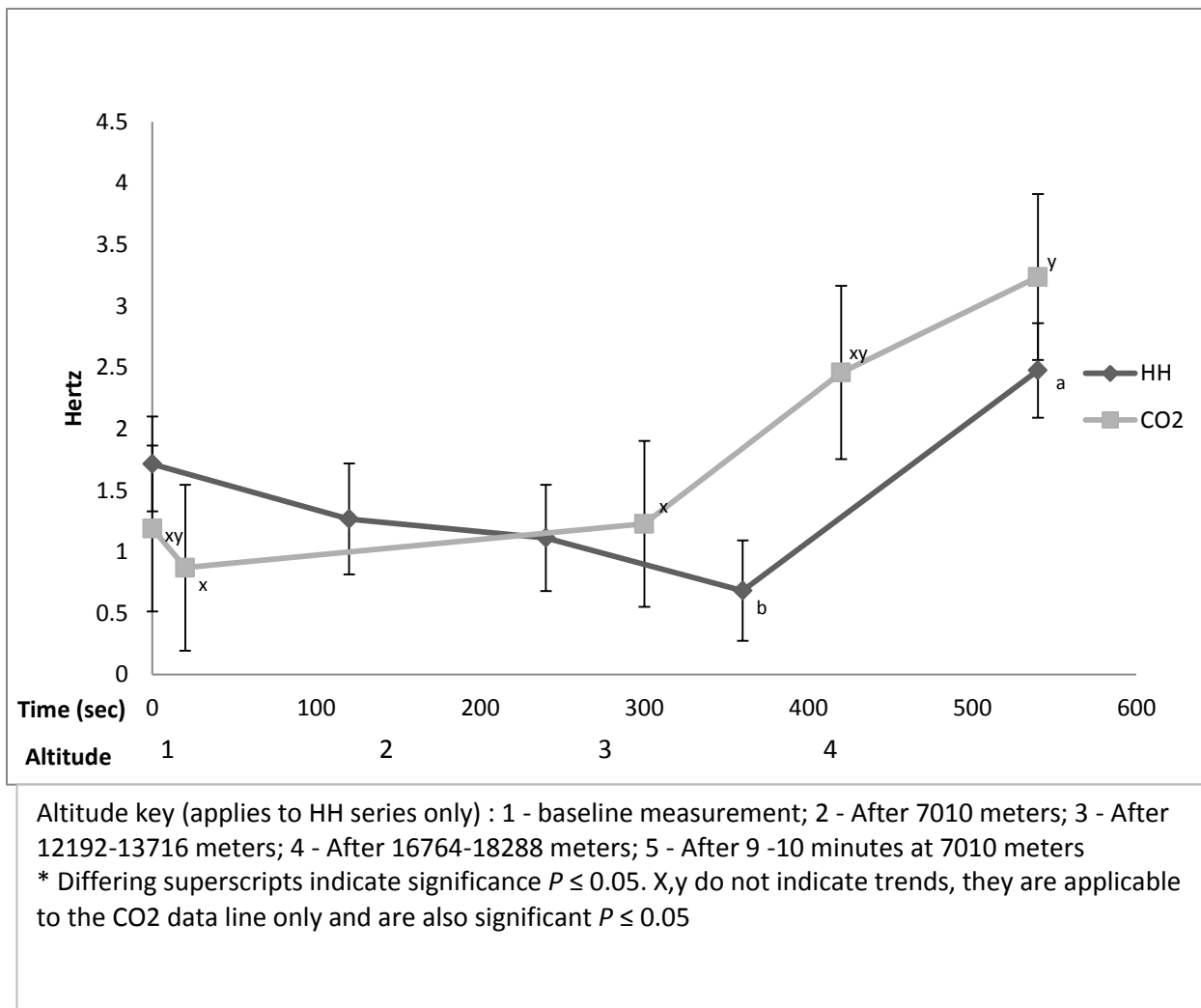
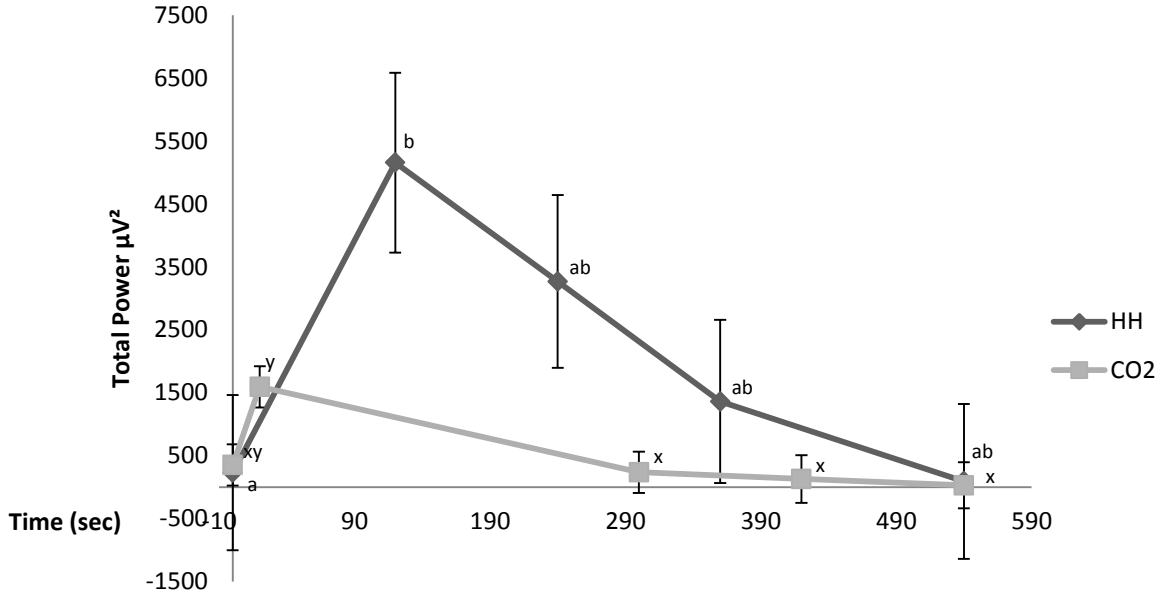


Figure 3-8. Electroencephalogram total power ( $\mu V^2$ ) values over time for both hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanized piglets (n= CO<sub>2</sub> – 9; HH – 6). Power measurements were not statistically compared between treatments but were plotted on the same time scale to provide for relative comparisons in treatments over time.



Altitude key (applies to HH series only) : 1 - baseline measurement; 2 - After 7010 meters; 3 - After 12192-13716 meters; 4 - After 16764-18288 meters; 5 - After 9 -10 minutes at 7010 meters  
 \* Differing superscripts indicate significance  $P \leq 0.05$ . X,y do not indicate trends, they are applicable to the CO2 data line only and are also significant  $P \leq 0.05$