ABSTRACT

MENDOZA BENAVIDES, SANTA MARIA. Dietary Supplementation of the Osmolyte Betaine to Swine Exposed to Heat Stress. (Under the direction of Dr. Eric van Heugten).

Pigs, in particular, are very susceptible to heat stress due to their limitation to dissipate metabolic heat. Heat stress in growing pigs causes slow growth and reduced carcass quality, while it reduces reproductive performance in sows. It is of great interest to evaluate dietary strategies to alleviate heat stress in pigs to maintain pork productivity during the summer season. Betaine is an osmolyte that helps to maintain water homeostasis and cell integrity, which are ideal properties to prevent the cascade of negative effects generated by hyperthermia. In study 1, the effects of dietary betaine (0, 0.1, 0.15, and 0.2%) on growing pig performance, serological and hematological indices during thermo-neutral and heat-stressed conditions were evaluated. Supplementation of betaine at 0.1% alleviated heat stress by improving feed efficiency and reducing rectal temperature, but did not improve ADG. Supplementation of betaine at 0.1 and 0.15% protected cardiac and skeletal muscles from tissue injury and maintained ion homeostasis in the acute phase of heat-stress, but not after prolonged heat stress. In study 2, two experiments were conducted to determine: 1) the effects of betaine (0.2%) in combination with ractopamine; and 2) the optimal betaine level (0, 0.0625, 0.125, and 0.1875%; and compared to a ractopamine control) for finishing pigs during heat stress. In Exp. 1 betaine at 0.2% reduced feed intake and pig growth, but did not impact feed efficiency or carcass characteristics, suggesting that the betaine level used was excessive. In Exp. 2, betaine up to 0.1875% did not affect pig growth or carcass characteristic. In both experiments, pigs fed ractopamine had substantially improved growth and carcass quality. The studies showed that betaine (0.0625 to 0.2%) was not an effective strategy to alleviate heat stress in finishing pigs.

In study 3, two experiments were conducted to evaluate the effects of dietary betaine on sow reproductive performance. The design was a 2 x 2 factorial arrangement, with dietary betaine at 0 or 0.2% and two periods of supplementation (lactation or post-weaning until 35 d post-insemination). In Exp. 1 (summer months), supplementation of betaine during lactation reduced feed intake and increased sow body weight losses, without affecting litter gain or the number of pigs weaned. Supplementation of betaine during the post-weaning period reduced weaning to estrus interval, regardless of parity group, and reduced farrowing rate in mature sows, but not young sows. Post-hoc analysis showed that betaine supplementation during lactation to parity 4, 5, and 6 sows increased total pigs born by 1.2 piglets, but reduced farrowing rate by 4.7% compared to control sows. For parity 1 sows, betaine supplementation in the post-weaning period increased the number of piglets born by 1.5, but reduced farrowing rate by 4.5%. Betaine did not affect litter size in parity 2 or 3 sows. In Exp. 2 (non-summer months), betaine did not affect sow or litter performance during the lactation period. Supplementation of betaine during lactation reduced weaning to estrus interval and farrowing rate in mature sows. Supplementation of betaine during the post-weaning period reduced total number of pigs born and pigs born alive, regardless of parity group.

Collectively, these studies demonstrated that supplementation of betaine to heat-stressed growing and finishing pigs did not improve growth performance, although betaine may slightly reduce the impact of heat stress during early exposure. In sows, betaine fed during the summer months reduced weaning to estrus interval and increased litter size in the subsequent production cycle. However, supplementation of betaine reduced feed intake and farrowing rate. Further research needs to be conducted in sows to determine if the detrimental effects are related to the level of betaine inclusion.

Dietary Supplementation of the Osmolyte Betaine to Swine Exposed to Heat Stress

by
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BIOGRAPHY

Santa Maria Mendoza Benavides was born on February 14, 1985, in Portoviejo, Ecuador to Luis Mendoza and Marcia Benavides. In Jan 2003, she was admitted to Zamorano University in Honduras. She spent four years to get her diploma in agriculture and animal science. She also had the opportunity to complete an internship at Vicosa State University and Lagoa Da Serra Ltda. in Brazil, where she gained experience in beef cattle nutrition and reproduction. After graduation, Murphy-Brown LLC (Laurinburg, NC) offered her a job opportunity to work as a manager trainee and supervisor of the breeding and gestation department on a sow farm. In spring 2011, she was admitted to pursue her MS in the Department of Animal Science at NCSU and graduated in December of 2012. Santa Maria continued with a Ph.D. in the same department and her research has focused on betaine as a dietary strategy to alleviate heat stress in swine.

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CHAPTER I: Literature Review

Introduction

The impact of seasonality in the swine industry generates economic losses in the amount of approximately US$ 300 million per year (St-Pierre et al., 2003). Reductions in pig growth rate and feed intake, increased number of days to market, and inferior carcass characteristics are commonly observed in pigs exposed to prolonged heat stress (Rhoads et al., 2013). Moreover, sow reproductive performance also declined during the summer season (St-Pierre et al., 2003). Heat stress decreased farrowing rate and the number of total pigs born (Bloemhof et al., 2013).

It is expected that due to global warming, very high temperatures are likely to occur more frequently (Melillo et al., 2014). The problem of heat stress is more pronounced in the current, modern swine industry, in which pigs have been genetically selected to produce more growth lean, leading to the selection of animals with greater metabolic heat production and greater susceptibility to heat (Brown-Brandl et al., 2001).

Heat stress has been shown to compromise gut barrier function and cause organ injury, which may predispose the pig to inflammatory responses (Sanz Fernandez et al., 2014). Understanding the impact of heat stress on swine performance and evaluating potential dietary strategies to reduce the impact of excessive heat allows maintaining viability and productivity under those circumstances.

Betaine is an osmolyte and has an important role in maintaining water homeostasis and cell integrity. Beneficial effects of betaine during heat stress have been demonstrated in poultry (Farooqi et al., 2005; He et al., 2015) and rabbits (Hassan et al., 2011).

The objectives of the present review are: 1) to review the mechanisms of heat adaptation by animals with special emphasis on livestock; 2) to review potential beneficial effects of betaine during heat stress condition; and 3) to compile evidence that betaine supplementation may be an effective strategy to minimize the impact of heat stress in pigs.

Mammalian Thermoregulation

When environmental temperature changes, activation of thermosensory neurons occurs. Thermosensory neurons are mainly located in the skin, viscera, spinal cord, and brain. Depending on their location, they can be classified as peripheral or central thermosensory neurons (Romanovsky, 2007). Neurons located in the skin and viscera are peripheral neurons with a subdivision of superficial or deep neurons, respectively. Neurons located in the spinal cord and brain are considered central neurons (Romanovsky, 2007).

In addition, thermosensory neurons can also be classified based on their activity. There are warm-sensitive and cold-sensitive neurons. Warm sensitive neurons are mainly located in the brain while cold sensitive neurons are mainly located in the skin, viscera, and spinal cord. Activation of warm-sensitive neurons triggers a heat defense while activation of cold-sensitive neuron triggers a cold defense (Romanovsky, 2007). Thermosensory neurons operate within a large temperature range and their activation and maximum response depends on temperature (Romanovsky, 2007). Therefore, extreme environmental temperatures will cause full activation of neurons to stimulate a cold or heat defense.

In mammals, the preoptic area (POA), which is located in the anterior hypothalamus region, is thought to function as the thermoregulatory center (Yousef, 1985a). Activation of thermosensory neurons sends impulses to the POA (Figure 1). The input of information to the

POA is termed the afferent pathway. Activation of thermosensory neurons in the POA sends impulses to activate a heat or cold defense (effector) and this output of information is termed the efferent pathway (Figure 1). Output and responses to stimuli can be distinguished by two categories: autonomic and behavioral (Romanovsky, 2007).

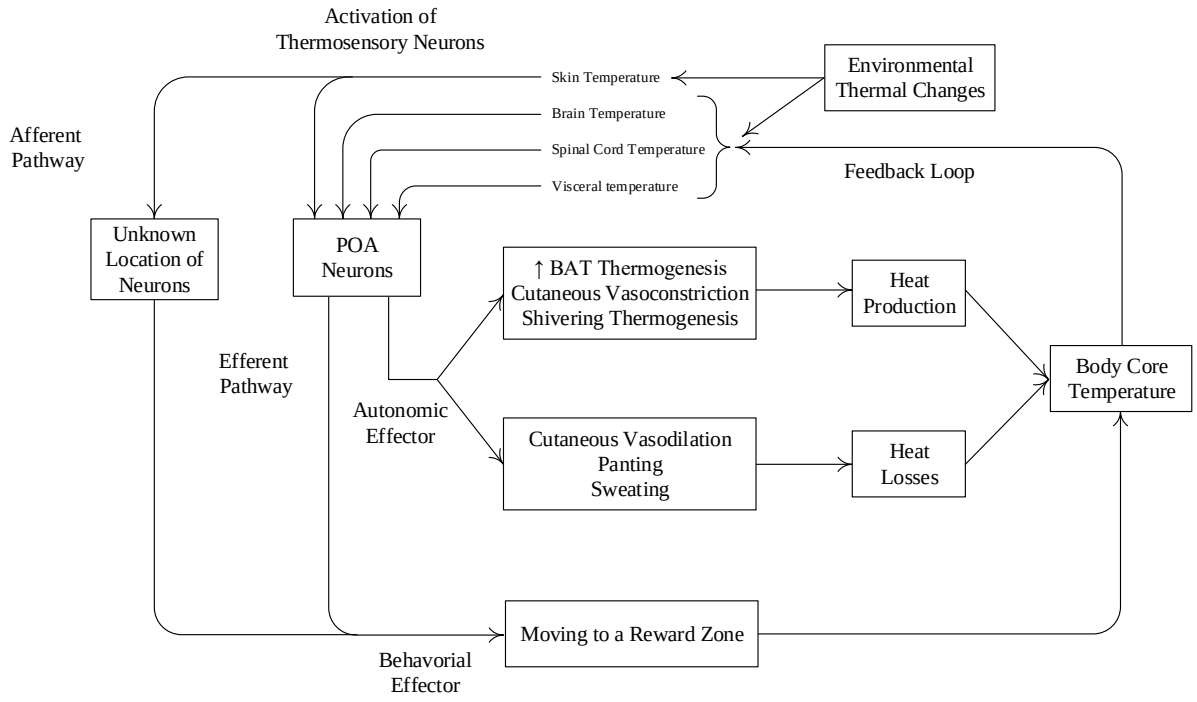

Figure 1. Diagram of the conceptual mammalian thermoregulation base in the rodent model. Adapted from (Nakamura, 2011). Environmental thermal changes are perceived by thermosensory neurons in the skin, brain, spinal cord, and viscera. Activation of thermosensory neurons sends impulses (afferent pathway) to the pre-optic area (POA) of the anterior hypothalamus. Activation of thermosensory neurons in the POA sends impulses (efferent pathway) to activate a heat or cold defense (effector). The behavioral effector is the self-movement of the animal to a reward zone. For the autonomic effector, a cold defense results in an increase of heat production in the brown adipose tissues (BAT), cutaneous vasoconstriction, and shivering. A heat defense causes cutaneous vasodilation, panting, and sweating. The effectors modify core body temperature, which is controlled by a feedback loop of the system.

Autonomic Effector

1. Heat Production

The autonomic process of heat production consists of increasing thermogenesis by the brown adipose tissues (BAT), cutaneous vasoconstriction (to prevent heat losses), and shivering thermogenesis (involuntary muscle contractions to generate heat) (Figure 1).

2. Heat Loss

The body can eliminate excess heat to the external environment via conduction, convection, radiant exchange, and evaporative cooling (Rubner, 1902). In order for this to occur, the external environment should be cooler than the body (Spiers, 2012). Conduction occurs when two objects are in contact, for example, lying down on cool surfaces. Convective heat transfer occurs when air near the skin absorbs the heat and then moves up from the surface (ventilation). Radiant exchange occurs by emission of heat to the surrounding objects and is disturbed by humidity (Spiers, 2012). Evaporative cooling is the mechanism of increased body water losses to carry heat to the environment.

Cutaneous vasodilation:

During heat stress animals increase blood flow to the skin through vasodilation of cutaneous blood vessels, allowing for increased heat dissipation from the skin. In that case, the surface temperature is similar to the internal body temperature (Yousef, 1985a). However, if the environmental temperature is greater than the skin temperature, the heat transfer through convection, conduction and radiation is not possible (Spiers, 2012). Instead, heat is dissipated

through evaporative cooling in which water from peripheral blood vessels is transferred to the skin surface and evaporates. (Houpt, 2004).

Panting and sweating:

Animals enhance evaporative cooling by panting and sweating. However, pigs do not have functional sweat glands (Spiers, 2012) and are incapable of sweating. The increase in respiration rate (panting) increases water loss because the air exhaled is saturated with water (Robertshaw, 2004). Usually, loss of water from the skin and respiratory tract is constant during thermo-neutral conditions, but it increases during heat stress (Houpt, 2004). Water can carry 586 cal/g of water evaporated at 20℃ (Yousef, 1985b). Pigs rely on evaporative cooling to dissipate metabolic heat. However, high environmental humidity can reduce heat transfer through evaporative cooling (Spiers, 2012).

Behavioral Effector

The behavioral effector of thermoregulation involves the movement of the whole body to a comfort zone. During heat exposure animals look for a breeze of cold air, shade, water and during cold exposure for a warm area or cuddling posture (Yousef, 1985a). Pigs in particular wallow in water or mud (Robertshaw, 2004), increase water intake (Patience et al., 2005) to compensate for water losses, and reduce feed intake (Renaudeau et al., 2011) to reduce metabolic heat due to activity of eating and digestion (Nienaber et al., 1996).

In the rodent model, evidence suggests that these behaviors do not require the POA, whereas autonomic effectors do (Almeida et al., 2006). It has been proposed that peripheral thermosensory neurons may have a direct effect on behavioral effectors, but the locations of

peripheral thermosensory neurons triggering behavioral effectors have not been identified (Romanovsky, 2007).

Thermo-neutral Zone of Swine

The thermo-neutral (TN) zone is defined as the range of ambient temperatures in which the body does not need to activate a cold or heat defense to prevent hypothermia or hyperthermia (Kingma et al., 2014). Consequently, there is more energy directed for animal growth and production (Spiers, 2012). Therefore, the TN zone has a lower and upper critical temperature, and these boundaries vary with the age of the pig. When the temperature exceeds the upper critical temperature, the heat defense pathway is activated. If cutaneous vasodilation is not sufficient to transfer heat to the environment, pigs increase evaporative heat losses to dissipate heat. In addition, evaporative heat losses can be discontinued if the humidity in the environment is high (Yousef, 1985a). When the temperature is below the lower critical temperature, the cold defense pathway is activated. Pigs increase thermogenesis by increasing muscle shivering because they lack BAT (Herping et al., 2002).

Lower and upper critical boundaries vary with the age of the pig. Heavy pigs have more difficulty dissipating heat because they have a relatively lower body surface compared to body mass than small pigs (Bruce and Clark, 1979). In contrast, small pigs are more susceptible to low environmental temperature. For instance, the TN zone for the lactating sow and piglet differ markedly; between 12 and 21°C for the sow (Black et al., 1993; Bloemhof et al., 2013) and between 30 and 37°C for the piglet (Mount, 1959; Black et al., 1993; Manno et al., 2005). The upper critical temperature of pigs decrease with increasing pig BW. For instance, the upper critical temperature for a 10 kg BW pig is 30°C, whereas it is 21°C for a 100 kg BW pig

(Renaudeau et al., 2011). Defining critical temperatures is of great interest for managing temperature settings in a commercial production environment to optimize productive performance.

Physiology of Heat Stress

Heat stress is the noticeable discomfort caused by high environmental temperatures that triggers physiological stress (Bouchama and Knochel, 2002). Living creatures have the ability to maintain the internal body temperature within a very narrow range (Gebremedhin, 2012). However, extreme environmental temperatures challenge adaptability of the animal.

When environmental temperatures exceed the upper critical temperature, pigs increase respiration rate and blood flow in the periphery to transfer metabolic heat to the environment. The loss of water and the redistribution of blood generate a cascade of negative effects like dehydration, organ injury, gut permeability, and inflammation (Cronjé, 2005). The cascade of events and the interrelationship of them with the physiological adaptation of animals to heat stress is shown in Figure 2 and will be discussed below.

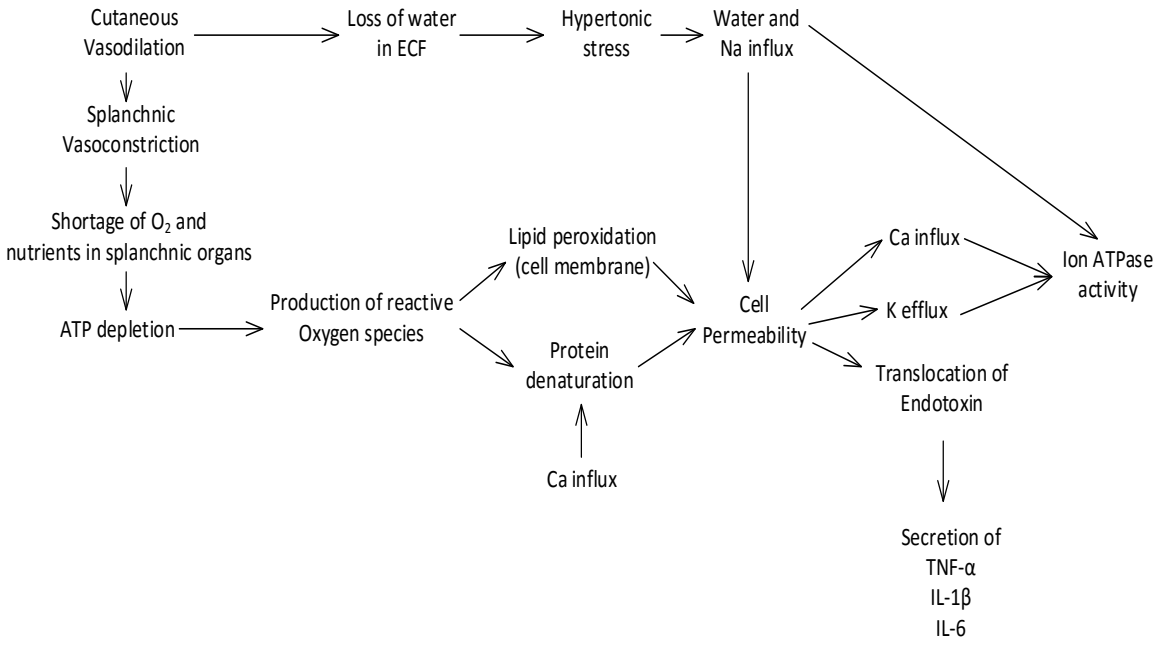

Figure 2. Cascade of negative effects due to heat stress adaptation. Adapted from Cronjé (2005). Extracellular fluid (ECF).

Dehydration

Fluids in the body are distributed into extracellular and intracellular compartments. The compartments are separated by the cellular membrane which is selectively permeable. Sodium is the major cation in the extracellular fluid and it is balanced by chloride and bicarbonate. The major cations in intracellular fluids are potassium and magnesium and are balanced by organic phosphates, sulfate, and proteins (Houpt, 2004). Ion homeostasis is extremely important for all cells because an excess or deficit of ions can cause disruption of the cell membrane and potentially cause cell death. Therefore, cells highly regulate the entry and exit of ions to control osmotic pressure and maintain cell integrity (van Vliet et al., 2001).

Heat stress increases water loss. In the body, the first compartment experiencing water loss is the extracellular fluids. Loss of water from the extracellular fluids will cause a rise in

sodium concentration (hypertonic stress). However, sodium concentration does not remain high during dehydration, instead, sodium is excreted from the body, and this prevents an increase in osmolarity. As dehydration continues, water from the intracellular fluids moves to the extracellular fluid, causing the excretion of potassium (Houpt, 2004). Sodium concentration in the extracellular fluids above normal stimulates the release of vasopressin (antidiuretic hormone) from the posterior pituitary. Vasopressin in the blood reduces urine volume and increases water intake (Houpt, 2004).

The concentration of sodium and potassium are carefully regulated by sodium/potassium ATPase. Sodium/potassium ATPase transports 3 sodium cations out of the cell while transporting 2 potassium cations into the cell, using ATP. The use of energy is required due to the fact that both cations are transported against their concentration gradients (Houpt, 2004). During heat stress the greater sodium concentration of extracellular fluid causes an influx of sodium and water into the intracellular space. This can cause a hypotonic stress and cell swelling. Sodium/potassium ATPase fails to restore ion homeostasis due to a deprivation of ATP. Regeneration of ATP cannot be accomplished due to splanchnic vasoconstriction that reduces the oxygen supply in heat-stressed animals (Cronjé, 2005).

Commonly, high serum osmolarity (concentration of solutes) indicates dehydration (Houpt, 2004). However, in pigs exposed to constant high environmental temperatures, loss of sodium through the urine or high water intake can cause serum osmolarity to be low (Patience et al., 2005). In this situation, animals may experience a hypotonic dehydration. Alternatively, hematocrit, which is the percentage of red blood cells relative to the total blood volume, can also indicate dehydration (Whalan, 2015).

Ischemia, Hypoxia and Oxidative stress

The body adapts by increasing the blood flow and volume in the periphery to dissipate metabolic heat. This causes a reduction in blood flow in the gut and splanchnic viscera (Cronjé, 2005). Sakurada and Hales (1998) reported an increase in blood flow by 28% in the heart, 42% in the metacarpal skin, 244% in the subcutaneous fat, and 315% in the peripheral fat of sheep exposed to heat. As a compensatory mechanism, heat-stressed sheep reduced blood flow by 31% in the brain, 27% in the kidney, 26% in the spleen, 58% in the stomach, and 16% in the ileum. This shortage of blood (ischemia) causes an inadequate supply of oxygen (hypoxia) and nutrients to the gastrointestinal organs, interfering with the regeneration of ATP (Cronjé, 2005). Moreover, the depletion of ATP impairs the activity of sodium/potassium ATPase and calcium ATPase. These enzymes maintain cellular ionic homeostasis. Greater accumulation of sodium inside the cell causes swelling and rupture of the cell membrane. Disruption of the cell membrane allows an unregulated calcium influx and potassium efflux, resulting in an increased demand for ion ATPase activity (Cronjé, 2005). The presence of reactive oxygen species (ROS) can attack the thiol group contained within calcium ATPase. Damage of the calcium ATPase, in particular, results in greater accumulation of calcium inside the cell which triggers the activity of proteases (Chihuailaf et al., 2002).

The simultaneous effects of hyperthermia and hypoxia contribute to the activation of cellular xanthine oxidase (Hall et al., 2001; Nanduri et al., 2014). Xanthine oxidase produces ROS such as superoxide ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO\cdot$). Reactive oxygen species can peroxidize lipids (Shahidi and Zhong, 2010) of the cell membrane and disrupt cell integrity, thereby increasing cellular permeability (Hall et al., 1999; Lambert

et al., 2002). The cascade of events caused by heat stress results in damage to the cells of gastrointestinal organs (Hall et al., 1999; Lambert et al., 2002) and accumulation of reactive oxygen species.

Organ Injury and Gut Permeability

Proteins or metabolites in the intracellular fluids are released into the transmembrane fluids and subsequently to the blood stream during severe heat stress. Abnormal concentrations of certain proteins in the plasma can provide information on which organs have been injured, depending on the type of proteins impacted. In addition, the relative increase of these proteins can provide information about the severity of organ injury (Franesoni and Mager, 1978). Heat stroke patients showed increased concentrations of plasma alanine transaminase (ALT) by 411%, aspartate transaminase (AST) by 590%, creatine phosphokinase (CPK) by 89% and lactate dehydrogenase (LDH) by 5% (Alzeer et al., 1997). Alanine transaminase and AST are mainly found in the liver, CPK in the skeletal and cardiac muscle, and LDH is found in most organs (Whalan, 2015). High levels of AST and a slight increase in ALT are indicators of myocardial infarction and high levels of AST and ALT are indicators of liver injury. High levels of plasma CPK indicate cardiac muscle, skeletal muscle, and brain injury and prolonged high levels of CPK indicate severe muscle damage (Whalan, 2015). Lactate dehydrogenase is considered the best predictor to determine the severity of heat exposure in humans (Alzeer et al., 1997). There are few studies conducted in heat-stressed pigs that have evaluated markers of tissues injury in the blood (Hicks et al., 1998; Pearce et al., 2013a). None of them reported changes in plasma CPK, ALT, or AST. In broilers, exposure to heat stress increased plasma CPK (Sandercock et al., 2001).

Cells lining the intestine are held together by a complex of proteins in the tight junctions (Balda et al., 1992). These proteins form a barrier to prevent the translocation of endotoxin from the intestinal lumen into the body (Cronjé, 2005). Endotoxin is a component of the outer membrane of most gram-negative bacteria that are also present in the microflora of the gut (Murphy, 2011). During high temperatures, the proteins of the tight junctions (occludin) are denatured (Dokladny et al., 2007), which allows the transport of endotoxin (e.g., lipopolysaccharide [LPS]) across the intestinal epithelium to the portal vein (Pals et al., 1997). Pigs exposed to heat stress have increased levels of endotoxin in the blood plasma and decreased electrical resistance of epithelial cells compared to pigs housed in thermo-neutral conditions. Consequently, exposure to heat stress causes increased gut permeability in pigs (Pearce et al., 2013b).

Inflammation

Presence of endotoxin in the blood stream initiates an inflammatory response. Cells of the immune system that express toll-like receptor (TLR)-4 are able to detect LPS and initiate an inflammatory response (Murphy, 2011). Macrophages can be activated by the presences of LPS and secrete ROS and nitric oxide (NO), antimicrobial substances that aim to destroy bacterial membranes (Murphy, 2011). Activated macrophages also secrete cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8. These cytokines are found in greater concentration in the blood plasma of patients exposed to high environmental temperatures (Robins et al., 1995). For instance, IL-1 induces fever, activates white blood cells, damages local tissue, and increases production of IL-6. TNF-α increases permeability of blood vessels and induces a fever response. IL-6 triggers hepatocytes to

produce acute phase proteins, such as C-reactive protein and mannose-binging lectin. Both bind to the surface of bacteria to enhance bacteria recognition by the complement system. IL-8 agglomerates neutrophils, basophils, and T-cell in the site of infection (Murphy, 2011). Consequently, translocation of endotoxin into the blood stream activates an unnecessary inflammatory response that diverts energy away from animal growth and productivity (Rakhshandeh and de Lange, 2012).

Energy Metabolism

Insulin is an anabolic hormone that promotes glucose uptake, glycogenesis, and fat storage and is particularly increased following food consumption. Heat-stressed animal have reduced feed intake, however, blood insulin concentration is increased despite the reduction in feed intake (Rhoads et al., 2013). This increase in insulin has been reported in heat-stressed cattle (O'Brien et al., 2010; Wheelock et al., 2010), pigs (Pearce et al., 2013a), and rats (Torlinska et at., 1987). The association of high levels of insulin during heat stress cannot be fully explained because heat stress largely stimulates catabolic processes, whereas insulin supports processes that are anabolic in nature (Rhoads et al., 2013). Although heat stress drastically reduces feed intake, research suggests that heat stress induces specific metabolic changes independent of its impact on feed intake, as evidenced in studies using pair-fed pigs housed under thermo-neutral conditions (Pearce et al., 2013). Indeed, heat stressed pigs have greater protein degradation, but do not increase lipid mobilization as compared to pair-fed control pigs. Heat-stressed sows (Prunier et al., 1997) and pigs (Pearce et al., 2013a) do not lose as much BW as their pair-fed thermo-neutral counterpart.

The use of glucose by the muscle is increased during heat stress (Fink et al., 1975; Jentjens et al., 2002). In thermo-neutral conditions, ingestion of carbohydrates reduces the release of glucose from the liver. In fact, the liver synthesizes glycogen for storage. However, during heat stress, the release of glucose by the liver continues, regardless of ingestion of carbohydrates (Angus et al., 2001). Heat stress increases glucose output by increasing glycogenolysis (Febbraio, 2001) and gluconeogenesis (Collins et al., 1980). In addition, plasma levels of lactate rise during heat stress, which has been reported in pigs (Hall et al., 1980) and humans (Fink et al., 1975). Lactate serves as a source for glucose production in the liver (Rhoads et al., 2013).

During heat stress, the muscle loses the ability to oxidize fat for energy, which is contradictory to the increase in fatty acid oxidation observed in pair-fed controls (Rhoads et al., 2013). Torlilnska et al. (1987) reported that heat-stressed rats have reduced plasma NEFA by 23% and reduced fat mobilization by 150% compared to thermo-neutral controls. This suggests that heat stress inhibits lipolysis (Torlilnska et al., 1987) independent of the reduction in feed intake caused by heat stress (Rhoads et al., 2013). Heat stress adaptation drives the body to accumulate more fat and reduce muscle, and changes in the carcass composition of pigs and poultry housed under high temperature conditions have confirmed these changes in energy partitioning over the years (Verstegen et al., 1973; Geraert et al., 1996).

Swine Productivity during Heat stress

Growing and Finishing pigs

Economic losses due to seasonality are close to US$ 300 million per year (St-Pierre et al., 2003). Renaudeau et al. (2011) showed that pig growth and feed intake gradually decreased when environmental temperatures increased. Pigs reduce feed intake as a defense mechanism to reduce heat increment (Renaudeau et al., 2011) that is caused by digestion and feeding activity (Nienaber et al., 1996). Collin et al. (2001) reported that pigs housed at 33°C reduced feed intake by 25% and such a reduction contributed to reducing heat production by 39% compared to pigs housed at 23°C.

The current genetic lines of swine have been selected for greater lean tissue and lower carcass fat deposition. Carcass yield percentage increased from 69.5% in 1960 to 73.9% in 2002, whereas lard yield reduced from 13.6% in 1960 to 1.9% in 1988. Consequently, lean percent increased by 1.55% between 1994 and 2004 (Brown-Brandl et al., 2004). Heat production increases with increased lean tissue accretion (Brown-Brandl et al., 2001) because protein turnover generates heat. Brown-Brandl et al. (2004) showed that heat production of current genotypes was as much as 30% higher than the estimates of Bond et al. (1959). The genetic selection of more productive pigs has also led to the selection of pigs with greater metabolic heat production associated with improved leanness; therefore, the modern genotype is more vulnerable to high environmental temperatures (Brown-Brandl et al., 2001).

It has been reported over the years that pigs raised during the summer had greater fat deposition and reduced lean (Verstegen et al., 1973; Bridges et al., 1998; Collin et al., 2001).

The changes in carcass composition are evidence of shifts in energy utilization of heat stressed animals (Geraert et al., 1996; Collin et al., 2001), which may be potential mechanisms of heat adaptation. However, such adaptations decrease carcass quality, thus reducing consumer preference and economic value. During the past decades, the pork industry has undergone a major transition from outdoor farms to more established indoor barns favoring both animals and farmers (Brown-Brandl et al., 2004). Enclosed facilities provide great benefits to food safety and animal welfare (Brown-Brandl et al., 2004), however, reduced space per pig limits the ability of pigs to dissipate heat, aggravating the problem.

Sow Performance

The lactating sow is highly sensitive to high environmental temperatures. Above 25°C, sows decrease feed intake by 0.5 kg/d per additional degree °C (Quiniou and Noblet, 1999). This reduction in feed intake causes greater sow BW losses, decreased milk yield, and reduced weight gain of the litter (Spencer et al., 2003). Sows exposed to 29°C lost 0.7 kg/d of body weight more than sows housed at 22° C, heat-stressed sows mobilized 40% more fat and lean tissue (Quiniou and Noblet, 1999). Sows with poor feed intake during the lactation period continue the subsequent reproductive cycle with a negative energy balance (Black et al., 1993).

The reduction in milk production due to heat stress is mainly explained by the reduction in feed intake. Baumgard et al., (2011) reported that dairy cattle exposed to heat stress reduced their feed intake by 23% and reduced milk production by 19% compared to cows housed in the thermo-neutral environment. In addition, pair-fed controls in that study had a reduction in milk production of 15%. Similar observations have been made in the lactating sow (Black et al., 1993). Sows exposed to 30°C reduced feed intake by 22% and milk

production by 16% compared to sows housed at 20 °C, while the pair-fed controls decreased milk production by 5% (Black et al., 1993). Consequently, heat-stress resulted in a reduction in milk production independent of the feed reduction associated with heat stress, which can be related to increased energy use for thermo-regulation or as a defense mechanism (Silanikove et al., 2009). In dairy cattle, β-casein fragment, a derivative of β-casein, binds to potassium channels on the apical side of the mammary epithelial and inhibits milk secretion. The β-casein fragment is primarily found in the milk of cows exposed to heat stress or another stressor (Silanikove et al., 2006; In sows, markers of milk inhibitors have not been studied yet. However, oxidative stress (a result of heat stress) decreased vitamin E and increased cytokines secretion in the milk of sows (Shen et al., 2015).

The negative effect of high environmental temperatures during the lactation period are carried over to the next reproductive cycle (Koketsu et al., 1996). Sows exposed to high environmental temperature have longer intervals between weaning and estrus or fail completely to exhibit estrus (Hennessy and Williamson, 1984). In addition, heat exposure before and after of the day of insemination compromised conception rate (St-Pierre et al., 2003), because the oocyte and sperm are sensitive to hyperthermia and oxidative stress (Hansen, 2012; Wolfenson and Thatcher, 2012). Furthermore, embryo losses are also increased by heat stress (Kojima et al., 1996). Consequently, heat stress reduced farrowing rate and the total number of pigs born, and increased non-productive days, with parity 1 sows being the most susceptible group (Bloemhof et al., 2013).

Decreased gut barrier function during heat stress may increase susceptibility to disease and cause inflammatory responses. The secretion of cytokines during inflammatory responses

has been shown to inhibit reproductive hormones. For instance, He et al. (2003) showed that LPS inhibits the activity of gonadotropin-releasing hormone (GnRH) neurons and reduces the secretion of luteinizing hormone (LH) in female rats. Evidence suggests that IL-1β is mainly responsible for the suppression of GnRH and LH secretion, but not follicular stimulating hormone (FSH) during endotoxin challenge (Tomaszewska-Zaremba and Herman, 2009). IL-1β increased COX-2/prostaglandin production by luteal cells, which impaired ovulation (Tomaszewska-Zaremba and Herman, 2009). Thus, the decline of sow reproductive performance during summer may also be caused by inflammation.

Betaine

Betaine or tri-methyl glycine is a source of methyl groups and an essential osmolyte that is found in plants and animals (Ashraf and Foolad, 2007; Lever and Slow, 2010). Betaine contains a quaternary ammonium, three methyl groups, and the alpha carbon is attached to the amine. Due to its molecular characteristics, betaine is considered a zwitterion. It is a neutral compound with a positive and negative charge. The osmolyte capacity of betaine may be due to its dipolar zwitterion characteristic and its high solubility in water (Chambers and Kumin, 1985).

Osmolyte Betaine

Betaine is found at relatively high concentrations in organisms that are adapted to drought, salinity, and extreme temperatures (Ashraf and Foolad, 2007). Cells of almost all organisms accumulate organic osmolytes when exposed to hyperosmolarity (Burg and Ferraris, 2008). In order to prevent intracellular water flux, cells increase inorganic ion (i.e.,

K^+, Na^+, and Cl^-) uptake (Strange, 2004). However, these ions can denature and precipitate cellular macromolecules (Sayed and Downing, 2011). Organic osmolytes, unlike electrolytes, do not affect the structure and functionality of macromolecules like proteins (Moeckel et al., 2002). Therefore, greater accumulation of betaine inside the cell can assure appropriate water volume during osmotic stress without impacting intracellular activity.

In plants, betaine is synthesized in the chloroplast via phosphatidylserine. Phosphatidylserine is converted to phosphatidylethanolamine by the enzyme phosphatidylserine decarboxylase, which is subsequently methylated by phosphoethanolamine N-methyltransferase and converted to phosphatidylcholine (Hanson and Scott, 1980). Choline is converted to betaine aldehyde in the chloroplast, by choline monooxygenase, which is then converted to betaine by betaine aldehyde dehydrogenase. Betaine synthesis can also occur by the methylation of glycine by the enzyme glycine N-methyltransferase. However, the major pathway for betaine synthesis is through choline oxidation (Weretilnyk et al., 1989).

In vertebrates, hyperosmotic stress increases betaine accumulation (Bagnasco et al., 1986; Nakanishi et al., 1990; Moeckel and Lien, 1997). The role of betaine as an osmolyte has important implications because it can maintain cell volume and integrity under challenging conditions. Indeed, betaine has been shown to improve performance in poultry when exposed to stress conditions such as coccidiosis infection (Klasing et al., 2002) and heat stress in broilers (Zulkifli et al., 2004; Farooqi et al., 2005) and rabbits (Hassan et al., 2011). Ion homeostasis is narrowly regulated; therefore, the body consumes a great amount of energy to keep osmotic balance. Betaine has demonstrated to have a sparing effect on sodium/potassium pump activity of 64% in erythrocytes (Moeckel et al., 2002). Cells of the intestine consume

25% of the total energy in the body (Huntington, 1999) due tohigh activity of the ion pumps. Therefore, the sparing effect of betaine on ion homeostasis can represent savings of 16% of the total energy consume by the body.

Other Organic Osmolytes

Microorganism like bacteria, archaea, and eukarya accumulate trehalose, proline, and betaine in response to hyperosmotic stress (Kempf and Bremer, 1998). In yeast, an increase in glycerol synthesis is observed in response of high external osmolarity (Påhlman et al., 2001). Under condition of high salinity, plants accumulate amino acids (proline and ectoine), sugars (glucose, fructose, sucrose, and fructans), sugar alcohols (mannitol and pinitol), polyamines (spermine and spermidine), quaternary amines (betaine and trigonelline), and pigments (carotenoids, anthocyanins, and betalains) as osmoprotectant mechanism (Parida and Das, 2005). In marine organisms, like mollusks and crustaceans, glycine, alanine, proline, taurine, betaine, and trimethylalanine oxide are found in great proportions in the tissues, while fish accumulate trimethylalanine oxide, taurine, alanine and glycine (Carr et al., 1996).

Osmolarity of the intracellular and extracellular fluid compartments are highly regulated to be in the range of 280 to 295 mosmol/kg in mammals. Therefore, red blood cells typically are not exposed to hyperosmotic stress (Burg and Ferraris, 2008). However, cells of the kidney are frequently surrounded by greater amounts of sodium chloride and urea, due to its functions in managing urine waste. These cells accumulate sorbitol, betaine, inositol, taurine, and glycerophosphocholine to prevent water efflux (Burg and Ferraris, 2008). Moreover, the brain and the liver may also experience hypertonic stress. The brain has a

preference to accumulate amino acids, choline, creatine, inositol, and taurine (Law, 1994), whereas liver cells accumulate betaine, inositol, and taurine (Haussinger, 1998).

Betaine Sources

Betaine is a common metabolite of living organism and can be found in many animal and plant feed products. Feed ingredients with high content of betaine are wheat and wheat products (Eklund et al., 2005), alfalfa, barley, and oats (Kidd et al., 1997). However, the most concentrated natural source is sugar beets that contain 1 to 1.5% betaine on DM basis. Beet molasses contains 3 to 8% betaine and approximately 60% sugar on DM basis (Heikkila et al., 1982). Betaine can be recovered by ion exchange chromatography (anhydrous or natural betaine of 97% purity), by crystallization as hydrochloride (betaine hydrochloride of 72% purity), or by extraction into organic solvents (Heikkila et al., 1982; Kidd et al., 1997). All these sources are commonly added to diets of poultry, swine, and cattle.

Betaine Uptake (Transporter)

Betaine is rapidly absorbed in the small intestine where it is transported by Na^+ independent transport systems or amino acid transport systems via active Na^+ coupled transport (Bro, 2008). There are two types of betaine transport systems via active Na^+ coupled transport: 1) neutral amino acids transporter and 2) imino acid carrier. Betaine shares these transporters with other amino acids. For instance, the neutral amino acid transporter can accept the neutral amino acids, proline, sarcosine, methylaminoisobutyrate, and glycine. The imino acid carrier can accept proline, alanine, and glycine (Munck, 1966).

In addition, there is the betaine GABA transporter (BGT)-1, which is a member of the brain GABA/norepinephrine transporter gene family (Yamauchi et al., 1992). The BGT-1 is Na^+- and Cl^--dependent and it is mainly found in the liver, kidney and brain. Betaine is actively accumulated into kidney cells by BGT-1, which is up-regulated when intake of sodium chloride is high (hypertonicity), via increased transcription of the transporter gene (Sheikh-Hamad et al., 1994; Burg, 1995).

Choline Oxidation in Mammals

In mammals, choline is found in lecithin (phosphatidylcholine) and sphingomyelin; both are essential components of cell membranes in the entire body (Kidd et al., 1997). Oxidation of choline into betaine is carried out in a two-step reaction. The enzymes choline dehydrogenase and betaine aldehyde dehydrogenase convert the hydroxyl group of choline into a carboxyl group. The electron acceptors of the reaction are FAD (flavin adenine dinucleotide) and NAD (nicotinamide adenine dinucleotide). The two enzymes are localized in the mitochondria (Chern and Pietruszko, 1999) and are only expressed in the liver and kidney (Flower et al., 1972).

Conversion of choline into betaine is limited by the transport of choline across the inner membrane of the mitochondria (Kaplan et al., 1993). The transporter has a saturable Km of 220 μM (Porter et al., 1992). There is insufficient information about the choline transporter in the mitochondria but it is suspected that it is a uniporter (passive transport) and that the hydroxyl and quaternary amine are key for binding (Porter et al., 1992). Once betaine is formed it is sent out of the mitochondria by passive diffusion and later to other organs (Lever and Slow, 2010). Porter et al. (1992) reported that betaine linearly accumulates over time when rat

liver mitochondria were cultured with 1 mM of choline. It has also been observed that human males supplemented with choline increased choline and betaine concentration in the blood plasma proportionally relative to the intake of choline (Veenema et al., 2008).

Betaine as a Methyl donor - Remethylation pathway

Homocysteine is a product of methionine catabolism. In the liver, synthesis and degradation of homocysteine are very well regulated. In the process of homocysteine formation, methionine adenosyltransferase catalyzes the formation of S-adenosyl methionine (SAM). SAM is the principal biological methyl donor, participates in the formation of epinephrine, creatine, phosphatidylcholine, and polyamine biosynthesis (Lu and Mato, 2005).

Homocysteine degradation or remethylation are determined by the levels of methionine. Sufficient levels of methionine elicit the conversion of homocysteine into cysteine for subsequent catabolism into sulfite for excretion. In contrast, low levels of methionine elicit the remethylation of homocysteine into methionine. Remethylation of homocysteine can be catalyzed by two enzymes: 1) Methionine synthase; this pathway is present in all tissues and depends on vitamin B_{12} and N^5-Methyl H_4 Folate (which donates the methyl group) as cofactors. 2) Betaine-homocysteine methyltransferase, which presumably is uniquely found in the liver; betaine donates a methyl group for methionine formation and liberates dimethylglycine (Finkelstein, 1990).

Supplementation of dietary betaine has been shown to increase the activity of betaine-homocysteine methyltransferase in rats (Finkelstein, 1983). Dietary betaine reduced plasma levels of homocysteine (Atkinson et al., 2008), increased SAM accumulation in the liver and

triggers the remethylation pathway (Barak et al., 1993). Consequently, betaine supplementation has a sparing effect on methionine (Finkelstein, 1990).

Betaine in Lipid and Protein Metabolism

Supplementation of betaine in diets of pigs (Cadogan et al., 1993; Lawrence et al., 2002; Rojas-Cano et al., 2011) and poultry (Hassan et al., 2005; Zhan et al., 2006; Xing et al. 2011) depressed overall fat deposition and increased lean deposition. Initially, it was believed that the mechanism by which betaine reduces fat deposition was via synthesis of carnitine (Huang et al., 2008). Carnitine is strictly required for transport of fatty acid inside the mitochondria for β-oxidation (Carter et al., 1995). Betaine can donate methyl groups for carnitine synthesis via SAM. Consequently, betaine could have increased fat oxidation by increasing the transport of fatty acids inside the mitochondria. But later Wray-Cahen et al. (2004) found that betaine did not increase fatty acid β-oxidation, but supplementation with carnitine did. Huang et al. (2008) found that betaine supplementation decreased the activity of acetyl-CoA carboxylase, fatty acid synthase (FAS), and malic enzyme in subcutaneous adipose tissue of pigs by 18.0%, 18.8%, and 14.5%, respectively. Additionally, betaine supplementation also increased the activity of hormone sensitive lipase and increased concentrations of NEFA were found in the plasma (Huang et al., 2006). This suggests that betaine can reduce lipogenesis and increase lipolysis via hormonal changes.

"Dietary betaine may influence lean deposition by stimulating the secretion of growth hormone (GH) and insulin-like growth factor (IGF)-1, enhancing the insulin receptor signaling pathway, increasing creatine synthesis, or by preventing muscle dehydration (protein denaturation) (Cholewa et al., 2014). In pigs, betaine supplementation (0.125% to finishing

pigs) increased serum basal concentrations of GH by 41%, total protein by 9%, and reduced urea nitrogen by 22% (Huang et al., 2007).

Betaine and Protein Denaturation

High temperatures cause proteins to lose functionality by interfering with the folding of the tertiary and secondary structure. Cells contain chaperones and proteases (heat shock proteins) that identify the proteins that can be repaired and the proteins that have excessive damage and should go towards proteolysis. Extreme high temperatures will trigger the proteolytic activities while mild temperatures will trigger repair activity (Spiess et al., 1999). Diamant et al. (2001) showed that *E. coli* cell cultures in a medium with betaine were protected from thermal (40 minutes at 44°C) protein denaturation compared to the control cultures without betaine. Betaine increased the rate at which heat shock proteins refolded proteins by up to 50%.

In addition, impaired activity of Ca-ATPase pump during heat stress increased Ca concentration inside the cell (Cronjé, 2005). Calcium activates proteases that break down the cytoskeleton (Chihuailaf et al., 2002). Cells cannot preserve shape and internal functionality. Erythrocytes culture in a medium with betaine have been shown to have reduced Ca-ATPase pump activity and sustain Ca balance. Betaine supplementation may be a potential strategy to prevent protein denaturation and increase protein refolding during heat stress.

Betaine Preserves Cell Integrity

Heat stress can cause leaky cell membranes (organ injury). Proteins or metabolites of the intracellular fluids are released into the transmembrane fluids and carried to the

bloodstream (plasma). In addition, leaky membranes of enterocytes can allow translocation of endotoxin of commensal bacteria and induce an inflammatory response (Cronjé, 2005).

Wettstein and Haussinger (1997) implemented a model of warm ischemia in rat livers to evaluate the osmoprotectant properties of betaine. The livers that were perfused with betaine and taurine showed reduced indicators of liver injury (-64% LDH and -52% AST) and reduced secretion of TNF-α by 36%. In another study, injection of LPS (10 mg/kg intraperitoneally) in rats pretreated with taurine and betaine (1.5%) in drinking water decreased AST, ALT, and hepatic malondialdehyde in plasma (Balkan et al., 2005). This study suggested that betaine (and taurine) prevent LPS-induced liver damage.

Betaine as an Antioxidant

The body is equipped with multiple antioxidant systems such as vitamin C, vitamin E, vitamin A, and also enzymes like glutathione peroxidase, catalase, superoxide dismutase and other peroxidases (Guérin et al., 2001). These systems aim to destroy free radicals (ROS) to prevent oxidative stress (accumulation ROS). Recently studies have shown the antioxidant properties of betaine. Alirezaei et al. (2014) used the rat model to test the antioxidant effect of betaine. They induced oxidative stress by providing ethanol and supplemented rats with 0 or 1.5% betaine in the diet. The activity of glutathione peroxidase and superoxide dismutase in the liver of rats increased by 100% and 13%, respectively. In addition, supplementation of betaine reduced thiobartituric acid reactive substance (TBARS), an indicator of lipid peroxidation, by 50%. Supplementation of betaine in the diet (1%) of poultry enhanced the antioxidant status in the breast muscle (Alirezaei et al., 2012). In that study, betaine increased

the activity of glutathione peroxidase, catalase, and superoxide dismutase by 56%, 38%, and 17%, respectively; and decreased TBARS by 40%.

Betaine and Embryo Development

Prior to implantation, embryos are very susceptible to perturbations of cell volume, even at osmolarity ranges that are considered to be physiologically normal. In the rodent model, it has been determined that the first signal of independence in fertilized oocytes is the activation of glycine transporter (GLYT1) and secondly betaine transporters (SIT1). The SIT1 is an imino transporter and unique in the fertilized oocyte. SIT1 is active during a short time; the maximum activity was reported to be between 4 and 10 h after fertilization when the fertilized oocyte reached the 2-cell stage. The activity declined after 30 hours when the fertilized oocyte reached the 4-cell stage (Anas et al., 2008). Both osmolytes, glycine, and betaine accumulated in great proportion in the rodent embryos (Baltz and Zhou, 2012).

The remethylation pathway of methionine through betaine may have two roles prior to implantation, osmoprotectant in the initial cellular division and later as a methyl donor in the blastocyst (Corbett et al., 2014).

In humans, high plasma concentration of homocysteine in pregnant women is associated with neural tube defect in the newborn and it can be prevented with supplementation of folate (Czeizel and Dudas, 1992). Folate is the cofactor of the enzyme methionine synthase, the pathway that co-exists with betaine-homocysteine methyltransferase, which methylates homocysteine. The importance of having sufficient carbon pool is extremely important during pregnancy, because high levels of homocysteine causes embryotoxicity (VanAerts et al., 1994) and embryo losses.

Dietary Supplementation of Betaine to Poultry

During coccidiosis infection, supplementation of betaine has shown to offset morphological changes in the small intestine by maintaining villus integrity and structure of the gut mucosa (Kettunen et al., 2001b; Klasing et al., 2002). Due to its function as an osmolyte, betaine may stabilize osmotic stress by minimizing water loss of the cells lining the gastrointestinal tract (Kettunen et al., 2001a; Klasing et al., 2002).

Betaine supplementation in the water (2.5 g/l) has shown to alleviate diarrhea and reduce litter moisture in turkey production systems (Ferket, 1995). During heat stress conditions, betaine supplemented in the water (50 g/l) to broilers reduced mortality by 10% (Zulkifli et al., 2004). Furthermore, under heat stress conditions, supplementation of betaine has shown to improve weight gain of broilers (Farooqi et al., 2005) and enhance egg production and eggshell quality in laying hens (Ryu et al., 2002).

Under normal conditions (no coccidiosis or heat stress), dietary supplementation of betaine (0.05 to 0.15%) resulted in increased ADG, gain:feed (Hassan et al., 2005; Zhan et al., 2006; El-Husseiny et al., 2007), breast yield (Waldroup and Fritts, 2005; Zhan et al., 2006), egg production (Lu and Zou, 2006), and egg weight (Park et al., 2006). On the other hand, others have reported no effect on growth (Waldroup and Fritts, 2005) or carcass quality (Schutte et al., 1997).

Dietary Supplementation of Betaine to Swine

In a meta-analysis, Sales (2011) reported that supplementation of betaine in diets of swine consistently increased G:F, carcass yield, and reduced back fat. The effect of betaine on

ADFI, lean percentage, and loin depth was variable and betaine did not impact ADG (Sales, 2011). Nonetheless, improvements in ADG due to betaine supplementation have been observed when pigs were fed energy-restricted diets (Schrama et al., 2003; Wray-Cahen et al., 2004; Dunshea et al., 2009). Betaine reduces maintenance energy requirements, attributable to osmotic regulation, and increases energy availability for growth (Schrama et al., 2003). Betaine supplementation has been reported to reduce ADFI (Matthews et al., 2001; Lawrence et al., 2002) in a dose dependent response. The beneficial effect of betaine might be limited by the level of inclusion (Eklund et al., 2005) and may depend on the dietary lysine:calorie ratio (Matthews et al., 1998). Haydon et al. (1995) reported that 0.10% betaine fed to gilts (103 kg of BW) reduced ADFI when diets contained 2.59, and 2.80 g total Lys/Mcal DE but not when diets contained 1.88 and 2.09 g total Lys/Mcal DE. Similarly, Matthews et al. (1998) reported that pigs (111 kg BW) fed diets containing 0.125% betaine reduced ADFI when diets contained 2.2 g SID Lys/Mcal ME but not when they contained 1.9 g SID Lys/Mcal ME.

The reduction of backfat due to betaine supplementation has been supported by the reduction of activity of lipogenic enzymes (Huang et al., 2008) and increase of hormone-sensitive lipases in adipose tissues of pigs fed betaine (Huang et al., 2006); the first favors fat degradation and the second reduces fat depositionand Yang et al. (2009) reported that betaine increased intramuscular fat in pigs. These new findings suggested that supplementation of betaine may cause a redistribution of lipid deposition rather than increase in fat oxidation (Sales, 2011). The effects of betaine on performance and carcass have been summarized by Eklund et al. (2005) and Ratriyanto et al. (2009) using studies from 1993 to 2008. The most

recent studies are summarized in Table 1. The studies also suggested that betaine affected carcass composition.

In sows, the effect of betaine has also been evaluated and showed positive effects on reproductive performance. Campbell and Virtanen (2001) offered 0, 0.1, 0.2, and 0.4% of betaine to lactating sows (parity 1 or parity 2+). Betaine at 0.4% reduced feed intake and consequently reduced litter growth. No effect on litter growth was observed when betaine was supplemented up to 0.2%. In addition, diets containing 0.4% betaine caused a reduction in farrowing rate in parity 1 sows. Betaine included at 0.2% improved total number of pigs born (+ 0.84 piglets) and pigs born alive (+1.13 piglets) in parity 1 and parity 2+. In a follow-up study, Campbell and Virtanen (2001) reported that parity 2+ sows were more responsive to supplementation of 0.2% betaine than parity 1 sows. Parity 2+ sows had increased total pigs born by 2.3 piglets while parity 1 did not show an increase.

Table 1. Effect of dietary betaine on performance and carcass characteristics of pigs

Animal	Betaine level, %	Betaine effect[1]	Reference
Gilts, 65 to 100 kg BW	0.2, 0.4, 0.6	↑ ADG and G:F 0.2% ↓ ADFI ↑ Betaine in tissue ↑ Muscle SFA ↓ Muscle USFA	(Yang et al., 2009)
Pigs, 55 to 90 kg BW	0.125	↑ BW and ADG ↑ Muscle fat ↑ Carcass yield ↓ Back fat	(Huang et al., 2009)
Gilts, 20 to 50 kg BW	0.5	↑ Lean ↓ Fat	(Rojas-Cano et al., 2011)
Barrows, 37 to 150 kg BW	0.1	No effect on growth ↑ intramuscular lipids	(Martins et al., 2012)

1 Average daily gain (ADG), gain:feed (G:F), saturated fatty acid (SFA), unsaturated fatty acids (USFA), body weight (BW).

Ramis et al. (2011) also evaluated betaine supplementation during lactation. Dietary betaine at 0.2% decreased feed intake compared to sow receiving 0% betaine (5.91 vs 5.43 kg), increased litter weight at weaning (51.26 vs. 57.35 kg), decreased weaning to estrus interval (5.7 vs. 4.7), increased pigs born alive in the subsequent cycle (13. 2 vs. 14.0 piglets), and increased the number of pigs weaned in the subsequent lactation cycle (10.5 vs.11.0 pigs). In a similar study, Greiner et al. (2014) reported that 0.2% betaine fed during lactation did not affect sow feed intake or litter gain. Consistently, betaine reduced weaning to estrus interval (7.9 vs. 4.9 d) and increased pigs total born in the subsequent cycle (9.8 vs. 12.6 pigs) compared to sows that did not receive betaine (Greiner et al., 2014). Van Wettere et al. (2012) reported that feeding betaine (7.6 to 9 g betaine/d) to gestating sows during summer months increased litter size in parity 3 sows or greater (13.6 vs. 12.1 piglets) compared to sows that did not receive betaine.

The mechanism of betaine increasing the number of pigs born is not fully understood. In the rodent model, it is suggested to be related to its osmoprotectant capacity and methyl donation for DNA formation (Corbett et al., 2014). In the human, the effect of dietary betaine is associated with a reduction of plasma homocysteine (Schwab et al., 2002; Hague, 2003).

Dietary Betaine and Heat Stress

Heat-stressed animals undergo dehydration, ion imbalance, hypoxia, ischemia in the splanchnic organs, increased oxidative stress, increased gut permeability, inflammation, and multi-organ injury. The common denominator of these events is the vulnerability of the cell membranes to lose integrity for which the osmolyte betaine has a protective effect. In addition, the properties of betaine in preventing protein denaturation, enhancing protein repair, and as antioxidant that may contribute to ameliorating the negative effects caused by heat stress have been discussed above. Below is a summary describing evidence why betaine can be an effective strategy to alleviate heat stress:

- Sparing effect on ion ATP pumps

  -Maintain ion balance (Moeckel et al., 2002)

  -Reduce ATP depletion (Moeckel et al., 2002; Schrama et al., 2003)
- Cell integrity

  -Prevent gut permeability (Klasing et al., 2002)

  -Reduce organ injury (Wettstein and Haussinger, 1997)

  -Reduce inflammation (Wettstein and Haussinger, 1997)
- Prevent protein denaturation (Moeckel et al., 2002; Chihuailaf et al., 2002)

- Enhance protein repair (Diamant et al., 2001)
- Reduce oxidative stress (Alirezaei et al., 2012; Alirezaei et al., 2014)
- Improve carcass characteristics (Huang et al., 2008; Sales, 2011; Cholewa et al., 2014)

Dietary Betaine Supplementation to Heat-Stressed Animals

Betaine supplementation in the water or in the diet of heat-stressed broilers, duck, and rabbits have been reported to improve growth performance, reduce mortality, reduce fat deposition, and improve parameters in the serum suggesting a reduction in lipogenesis and organ injury. The studies are summarized in Table 2. The effect of betaine in heat-stressed growing pigs have not yet been evaluated. However, under thermo-neutral conditions, supplementation of betaine to pigs reduced fat deposition (Lawrence et al., 2002; Huang et al., 2006) and improved growth when pigs were fed energy-restricted diets (Schrama et al., 2003; Dunshea et al., 2009). Reduced energy intake occurs in pigs housed under heat stressed conditions, which may provide an opportunity for betaine to exert positive effects on production performance. In addition, betaine supplementation to lactating and gestating sows has shown to increase litter size particularly in older sows during summer months (Campbell and Virtanen, 2001; van Wettere et al., 2012).

Together these data provide compelling arguments that betaine supplementation may be an effective strategy to minimize the impact of heat stress in growing pigs and sows. We further propose that the multiple properties of betaine can provide beneficial effects at several critical points in the progression of heat stress contributing to effectively improve pig and sow performance.

Table 2. Effects of betaine supplementation to heat-stressed animals

Animal	Betaine level, %	Temp., °C	Betaine effect	Reference
Broilers	5% in water	35	↓ Water intake ↓ Mortality	(Zulkifli et al., 2004)
Broilers	0.1% in diet	32 - 35	↑ ADG, ADFI	(Farooqi et al., 2005)
Rabbits	0.0, 0.025, 0.05, 0.075, 0.1% in the diet	30	↑ ADG, ADFI, G:F, HCW ↓ RT, RR ↑ Serum: Protein, Lipids, K^+, P, T_3 ↓ Serum: Alk P, Glucose, Na^+	(Hassan et al., 2011)
Broilers	0.0, 0.05, 0.1% in water	32	↑ BW, G:F ↓ ADFI	(Sayed and Downing, 2011)
Ducks	0.0, 0.05, 0.1, 0.15% in diet	Summer	↑ BW, ADG, G:F ↓ ADFI, Mortality ↑ Serum: Protein ↓ Serum: Lipid, TG, AST, ALT	(Awad et al., 2014)
Broilers	0.1% in diet	32	↑ ADG, ADFI ↓ Abdominal fat	(He et al., 2015)
Broilers	0.1% in diet	33	↑ BW	(Akhavan-Salamat and Ghasemi, 2016)

[1] Average daily gain (ADG), average daily feed intake (ADFI), gain:feed (G:F), rectal temperature (RT), respiration rate (RR), triiodothyronine (T_3), alkaline phosphatase (Alk P), body weight (BW), triglycerides (TG), aspartate transaminase (AST), alanine transaminase (ALT).

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CHAPTER II:

Effects of dietary supplementation of the osmolyte betaine on growing pig performance, serological and hematological indices during thermo-neutral and heat-stressed conditions

ABSTRACT: The present study was designed to evaluate the effects of dietary betaine on pig performance, serological and hematological indices during thermo-neutral and heat-stressed conditions. Pigs (n=64; BW 39.0±1.5) were assigned within weight blocks and sex to 1 of 8 treatments and housed in individual pens. Treatments consisted of 2 environmental temperatures (thermo-neutral or heat-stressed) and 4 levels of betaine (0.00, 0.10, 0.15, and 0.20%). Room temperatures followed a daily pattern with a low of 14 and a high of 21°C for the thermo-neutral environment and a low of 28 and high of 35°C for the heat-stressed environment. Diets were corn-DDGS-soybean meal based (2.96 g SID lysine/Mcal ME) and were fed for 7 d (constant 21°C) prior to imposing temperature treatments. Respiration rate and rectal temperature were measured on d 0, 1, 2, 3, 7, 14, 21 and 28. Data were analyzed using the Glimmix procedure of SAS, weight block nested within environmental temperature was used as a random effect. Day of measurement was analyzed as repeated measure. Heat stress reduced ($P \leq 0.008$) ADG (0.710 vs. 0.822 kg/d) and ADFI (2.27 vs. 1.81 kg/d), and increased G:F ($P=0.036$; 0.391 vs. 0.365) for the 28-d period. Betaine increased G:F quadraticly ($P=0.071$; 0.377, 0.391, 0.379, and 0.366) with the greatest response at the 0.10%, regardless of the environment. Heat stress increased ($P \leq 0.001$) respiration rate and rectal temperature throughout d 1 to 28 (23 vs. 48 breaths/30s, and 38.94 vs. 39.47°C). Betaine at 0.10% tended to reduce rectal temperature to heat-stressed pigs ($P=0.078$). Heat stress increased K and creatinine ($P<0.001$). Heat stress increased creatine phosphokinase (CPK) and reduced Na on d 3 (interaction, $P<0.001$). Betaine fed at 0.15% decreased K on d 3 (interaction, $P=0.04$) and reduced CPK on d 3 only to heat-stressed pigs (interaction, $P=0.03$). Heat stress did not impact hematological indices ($P>0.13$). Heat stress reduced growth, disturbed ion

balance, and caused muscle injury. Betaine had a minor impact alleviating heat stress in the early days of heat exposure. The beneficial effect of betaine on prolonged heat exposure was diminished by pig adaptation to heat.

Key words: pigs, betaine, heat stress.

Introduction

High ambient temperatures account for US$ 330 million per year in losses for swine producers in the United States (St-Pierre et al., 2003). The temperature worldwide is forecasted to continue to rise (Turral et al., 2009), which implies that production losses are expected to increase (Nelson et al., 2009). Heat-stressed pigs have increased mortality, and reduced growth, gain:feed, market weight, and carcass value (Rhoads et al., 2013).

During heat stress pigs adapt by increasing cutaneous vasodilation as a defense mechanism to dissipate metabolic heat to the environment. This mechanism is compensated by a reduction in blood flow to the splanchnic organs, causing a shortage of nutrients and oxygen. In the absence of oxygen, cells are depleted of ATP and accumulate reactive oxygen species (ROS). Reactive oxygen species disrupt the cell membrane affecting ion homeostasis and increasing the demand for ion ATPase activity (Cronjé, 2005). Consequently, heat stress promotes cell damage, especially in enterocytes and hepatocytes (Hall et al., 1999). Disruption of the cells lining the intestine lead to the passage of endotoxin from commensal bacteria (Lambert et al., 2002; Pearce et al., 2013a) causing an inflammatory response.

Betaine or tri-methyl glycine is known for its methyl donor and osmolyte properties (Kidd et al., 1997; Craig, 2004). Betaine is especially found in plants that are adapted to drought, salinity, and extreme temperatures (Ashraf and Foolad, 2007). As an osmolyte,

betaine has been demonstrated to reduce the activity of ion ATPase pump of the erythrocyte (Moeckel et al., 2002) and to maintain ion balance. In addition, betaine prevented liver injury and endotoxin translocation in heat-stressed rats (Wettstein and Haussinger, 1997) and prevented villi damage during coccidiosis infection in poultry (Kettunen et al., 2001; Klasing et al., 2002).

Dietary betaine increased growth and reduced hyperthermia of heat-stressed rabbits (Hassan et al., 2011) and increased growth (Farooqi et al., 2005; Sayed and Downing, 2011; He et al., 2015) and reduced mortality (Zulkifli et al., 2004; Awad et al., 2014) in heat-stressed poultry. In pigs, betaine improved pig performance during feed restriction (Schrama et al., 2003; Dunshea et al., 2009) due to a reduction in energy requirements for maintenance associated with energy savings of ATPase pump activity in the cells of the gastrointestinal organs (Schrama et al., 2003).

We hypothesized that the osmolyte betaine could ameliorate the negative effects of high environmental temperatures. The objective of this study was to evaluate the impact of dietary betaine on pig performance, core body temperature and respiration rate, and serological and hematological indices during thermo-neutral and heat-stressed conditions.

Materials and Methods

Animal use protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee.

The experiment was conducted using 64 crossbred ([Landrace x Yorkshire] x [Hampshire x Duroc]) pigs (16 barrows and 48 gilts), with an average initial BW of 39.0±1.5

kg. Pigs were blocked by initial BW and sex and assigned to 1 of 8 treatments using an experimental animal allotment program (Kim and Lindemann, 2007). Treatments consisted of two thermal environments (thermo-neutral or heat-stressed) and 4 levels of dietary betaine (0, 0.10, 0.15, and 0.20%) (Betafin®, Danisco A/S, Marlborough, Wiltshire, United Kingdom).

Two identical rooms were used, each equipped with an environmental control system (GT-5124LW Grower Direct, Monitrol Inc., Boucherville, Quebec, Canada) allowing temperatures to fluctuate over time to mimic those experienced in commercial production systems. One room was used as the thermo-neutral room and temperatures were set at 15, 14, 15, 15, 17, 18, 19, 20, 21, 20, 17, and 15°C, for 2400, 0200, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200 h, respectively. The other room served as the heat-stressed environment and temperatures were set at 29, 28, 29, 29, 31, 32, 33, 34, 35, 34, 31, and 29°C for 2400, 0200, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200 h, respectively. Room temperature was recorded by data recorders (Logtag, MicroDAQ Ltd., Contoocook, NH) every 10 min. Two data recorders were placed in each room at approximately the same height of the pigs.

Pigs were housed in individual pens (0.91 x 1.82 m) at the Swine Educational Unit (Raleigh, NC) using 32 pens in the thermo-neutral room and 32 pens in the heat-stressed room. Each pen had one nipple water drinker and a single space feeder. Feed and water were provided to allow ad libitum access throughout the entire experiment. Each room was equipped with a water meter to measure water disappearance in the room.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit (Raleigh, NC). Diets were corn-DDGS-soybean meal based and contained 2.96 g

standardized ileal digestible lysine/Mcal ME (Table 1). Diets were formulated to meet or exceed all nutrient concentrations suggested by NRC (2012). One basal mix was prepared to contain all ingredients, except betaine. This mix was then divided into 4 batches and each batch was mixed with the appropriate amount of betaine to generate the final dietary treatments. Pigs were fed dietary treatments for 7 d prior to initiation of temperature treatments. The temperature during this period was set at a constant 21°C and this was followed by the implementation of the 2 environmental treatments for a period of 28 d.

Pig BW was measured on d -7, 0, 14, and 28. Feed disappearance was measured from the difference of daily feed additions and feed refusal. Feed refusal was recorded on d 0, 14, and 28. Measurements were used to calculated pig ADG, ADFI, and G:F. Rectal temperature and respiration rate were measured on d 0, 1, 2, 3, 7, 14, 21, and 28, between 1400 and 1700 h. Measurements on d 0 were collected prior to exposure to environmental temperature treatments and served as baseline measurements. Rectal temperature was measured by using a digital thermometer (M750 Series, GLA Agriculture Electronics, San Luis Obispo, CA). Respiration rate was measured as the number of flank movements per 30 s. On d 3 and 28, blood was collected from the jugular vein by venipuncture in glass vacuum tubes without additive (for serum) and with K_3-EDTA (for whole blood). Blood was centrifuged at 1,000 × g for 20 min at 10°C to collect serum.

Chemical Analysis

The content of anhydrous betaine in the final diets was analyzed by Eurofins (Eurofins Scientific Inc., Des Moines, IA) using capillary electrophoresis. Dietary lysine, glycine, and methionine concentrations were measured by Ajinomoto Heartland, Inc. (Chicago, IL) using

ion exchange chromatography (Method 13903; ISO, 2005). Proximate analysis was conducted by the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC (2005) procedures. Diets were analyzed for CP (Method 990.03), crude fat (Method 920.39 (A)), crude fiber (Method 978.10), Ca (Method 985.01 (A, B, D)) and P (Method 966.01).

Serum samples were analyzed for total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK P), γ-glutamyltranspeptidase (GGTP), urea N, creatinine, glucose, Ca, P, Mg, K, Na, Cl, cholesterol, triglycerides, non-esterified fatty acids (NEFA), amylase, lipase, creatine phosphokinase (CPK), free cysteine, free methionine, and homocysteine. Whole blood was analyzed for white blood cell count (WBC), red blood cell count (RBC), hemoglobin, hematocrit, platelet count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. All analyzes were performed by Antech Diagnostics Laboratory (Lake Success, NY) using an autoanalyzer (Olympus AU 5400, Olympus America Inc., Melville, N.Y.) except for NEFA, cysteine, methionine, and homocysteine. Non-esterified fatty acids concentration were determined by a commercial kit (NEFA-HR2 kit, Wako, Mountain view, CA) following the manufacturer's protocol (Wako Diagnostics, 2012). Cysteine, methionine, and homocysteine were measured by HPLC using an autoanalyzer (Hitachi L8900, Hitachi High Technologies America, Inc., Dallas, TX) following the procedures of OJEU (1998) (Method, L257/16). Osmolarity was calculated using the equation given by Tormey (1997) as follows:

$$\text{Serum Osmolarity (mOsm per L)} = 2[\text{Na (mEq per L)} + \text{K (mEq per L)}]$$

$$+\left[\frac{\text{Glucose (mg per dL)}}{18}\right] + \left[\frac{\text{urea N (mg per dL)}}{2.8}\right]$$

Statistical Analyses

Data were analyzed using the Glimmix procedure of SAS (SAS Inst. Inc., Cary, NC). The normality of the residuals was tested using the Univariate procedure of SAS (Shapiro-Wilk test) (Shapiro and Wilk, 1965) and considered normal at $P \geq 0.10$. When the residuals did not have a normal distribution, data were transformed to log normal. The pig was used as the experimental unit. For pig performance, the model tested for fixed effects of betaine, environment, and their interaction; block nested within environment was used as the random effect.

Measurements of rectal temperature and respiration rate on d 0 (prior to initiation of temperature treatments) were evaluated to detect potential effects of the room, betaine, and their interaction. Measurements of rectal temperature and respiration from d 1 to 28 were analyzed to test the fixed effects of betaine, environment, day of measurement, their interactions, and values of d 0 were used as a covariate when significant ($P \geq 0.05$). Day of measurement was analyzed as repeated measurements and the covariance structure was selected base in lowest Akaike's Information Criteria (AIC) (Kincaid, 2005). Compound symmetry and unstructured covariance structures were the most suitable according to the AIC for rectal temperature and respiration rate, respectively. In addition, respiration rate was transformed to log normal. Linear and quadratic contrasts were conducted to evaluate the changes over time of rectal temperature and respiration rate.

For blood chemistry and blood cell count, the model tested for fixed effects of betaine, environment, day of collection, and their interactions. Data were transformed to log normal for GGTP, creatinine, glucose, triglycerides, NEFA, lipase, CPK, WBC, RBC, neutrophils, lymphocytes, eosinophils, and basophils. Block nested within environment was used as the random effect.

Least squares means were reported, and differences were considered statistically significant at $P \leq 0.05$ and were considered tendencies when $0.05 < P \leq 0.10$. Orthogonal contrast comparisons were conducted to determine linear and quadratic effects of betaine level. Least squares means were compared using Tukey's method (Tukey, 1953).

Results

Room Temperature

The average room temperature during d -7 to d 0 was 21.02 ± 0.66°C and 21.61± 0.53°C for the rooms used as thermo-neutral and heat-stressed environment, respectively (data not shown). During d 0 to d 28 average temperature was 19.27 ± 2.19 °C and 31 ± 3.14 °C for the thermo-neutral and heat-stressed rooms, respectively (Figure 1). Water disappearance was 6.3 and 17.9 L per pig per day from d 0 to 28 for the thermo-neutral and heat-stressed environment, respectively.

Pig Performance

During the course of the experiment, 1 pig was euthanized on d -5 due to a prolapse, and 1 pig was removed on d 6 due to poor feed intake. Both pigs were in the room used as the

heat-stressed environment and were receiving diets containing 0.10 and 0.15% betaine, respectively. No differences in performance were observed during d -7 to d 0 (Table 2). There were no interactive effects between environmental temperature and betaine on growth performance. Exposure to the heat-stress reduced ($P \leq 0.008$) ADG, ADFI, and increased ($P = 0.036$) G:F during d 1 to 28. The increase in G:F due to heat stress was most pronounced during d 1 to 14 ($P = 0.014$). Betaine tended to increase G:F ($P = 0.071$) in a quadratic manner with the greatest response at the 0.10% inclusion level, regardless of environmental temperature.

Rectal Temperature and Respiration Rate

Rectal temperature and respiration rate did not differ between rooms ($P > 0.28$) or due to betaine supplementation ($P > 0.31$) prior to imposing environmental temperature treatments (d -7 to 0). Pig rectal temperatures were 39.18 and 39.13°C (SEM = 0.05) for the rooms used as thermo-neutral and heat-stressed, respectively. Rectal temperature was 39.16, 39.09, 39.11, and 39.26°C (SEM = 0.07) for 0, 0.10, 0.15, and 0.20% of betaine inclusion, respectively. Respiration rate during d -7 to d 0 was 14.8 and 15.9 (SEM = 0.7) flanks movements/30 s for the rooms used as thermo-neutral and heat-stressed, respectively. Respiration rate was 15.1, 14.9, 15.2, and 16.4 flanks movements/30s (SEM = 1.0) for 0.00, 0.10, 0.15, and 0.20% of betaine inclusion, respectively.

Exposure to heat stress increased ($P < 0.001$) rectal temperature, and this increase was more evident (interaction, $P < 0.001$) on d 1 of heat exposure (Figure 2A). Rectal temperature decreased linearly ($P \leq 0.001$) with increasing days on test, regardless of the environment. Exposure to heat stress increased ($P < 0.001$) respiration rate and this increase was more evident (interaction, $P < 0.001$) on d 1 and 3 of heat exposure (Figure 2B). Respiration rate

measured on d 28 of heat stress was similar to the respiration rate of pigs housed in the thermo-neutral room on d 7. Respiration rate linearly decreased ($P \leq 0.001$) as time progressed, regardless of the environment.

Betaine supplementation tended to reduce rectal temperature in a quadratic manner ($P = 0.071$) in pigs housed in the thermo-neutral environment, whereas betaine supplementation at 0.10% reduced ($P = 0.078$) rectal temperature in pigs housed in the heat-stressed environment (interaction, $P = 0.055$) (Figure 3A). Betaine supplementation linearly increased ($P = 0.037$) respiration rate in pigs housed in the thermo-neutral environment and linearly reduced ($P = 0.067$) respiration rate in heat-stressed pigs (interaction, $P = 0.040$) (Figure 3B).

Serum Chemistry

Serum chemistry panels were conducted on d 3 and 28 to evaluate the impact of acute and chronic heat stress, respectively. Heat stress significantly impacted many serum chemistry parameters and the impact of heat stress depended on whether it was measured during the early or late stages of heat stress (Table 3, 4, and 5).

Pig housed in a heat-stressed environment had increased concentrations of serum globulin, triglycerides, and cysteine, regardless of the day of measurement ($P \leq 0.048$). Heat stress reduced serum Ca^{++} concentrations ($P = 0.002$), activity of lipase ($P < 0.001$), serum A/G ratio ($P \leq 0.014$), and serum Na/K ratio ($P < 0.001$). Serum Ca^{++}, lipase activity and serum A/G ratio were lower on d 28 regardless of the environment ($P < 0.011$), whereas serum Na/K ratio was greater on d 28 regardless of the environment ($P = 0.008$).

Pigs housed in the heat-stressed environment had reduced serum ALT activity and NEFA concentration on d 28, whereas pigs in the thermo-neutral environment had increased

serum ALT activity and NEFA concentrations on d 28; ALT and NEFA levels were similar for heat-stressed and thermo-neutral pigs on d 3 (interaction, $P \leq 0.011$). Heat stress increased serum activity of AST on d 3; however, serum AST activity of heat-stressed pigs on d 28 was similar to the pigs housed in the thermo-neutral environment (interaction, $P = 0.034$). Heat-stressed pigs had reduced serum concentrations of urea N, Na^{+}, and homocysteine compared to pigs housed in the thermo-neutral environment on d 3, but they did not differ on d 28 (interaction, $P \leq 0.013$). Heat stress increased serum Mg^{++} concentration compared to pigs housed under thermo-neutral conditions on d 28, but this was not the case on d 3 (interaction, $P < 0.001$).

Serum methionine concentration was reduced due to heat-stress and this effect was more pronounce on d 3 (interaction, $P < 0.001$). Serum urea N/creatinine ratio concentration was reduced due to heat-stress and this effect was more pronounce on d 28 (interaction, $P = 0.045$). Serum activity of GGTP was increased on d 28 when pigs were housed under thermo-neutral conditions, and such a level was similar to the serum GGTP activity in pigs housed under heat-stressed conditions (interaction, $P = 0.034$).

Moreover, betaine supplementation reduced serum concentration of albumin in a quadratic manner ($P = 0.005$), showing the lowest level at 0.10% betaine. This reduction was more pronounced on d 3 regardless of the environment ($P < 0.001$).

Heat stress increased serum K^{+} serum concentrations ($P < 0.001$) and serum K^{+} concentrations were reduced on d 28 regardless of the environment ($P = 0.008$). An interaction between day of measurement and betaine was observed for K^{+} serum concentrations ($P = 0.041$). Supplementation of betaine decreased serum K^{+} concentration linearly ($P = 0.019$) on

d 3, but not on d 28, regardless of the environment. Heat stress reduced osmolarity on d 3 but did not affect osmolarity on d 28 (interaction, $P = 0.001$). In addition, supplementation of betaine tended to linearly decrease osmolarity ($P = 0.100$) when pigs were housed under thermo-neutral conditions and quadratically decreased osmolarity ($P = 0.013$) when pigs were housed under heat-stressed conditions, showing the lowest osmolarity value at 0.10% betaine, irrespective of the day of measurement (interaction, $P = 0.072$) (Figure 4).

Serum activity of ALK P was reduced due to heat stress ($P < 0.001$) and serum activity of ALK P was reduced on d 28 compared to d 3, regardless of the environment ($P < 0.001$). An interaction between environment and betaine were observed for serum activity of ALK P ($P = 0.015$). Supplementation of betaine during heat stress decreased serum activity of ALK P in a quadratic manner ($P < 0.001$), showing the lowest ALK P activity at 0.10% betaine. Betaine supplementation did not affect ALK P in pigs housed under thermo-neutral conditions.

Serum creatinine concentration was greater in heat-stressed pigs ($P < 0.001$) and serum creatinine concentration increased on d 28 as compared to d 3, regardless of the environment ($P < 0.001$). An interaction between environment and betaine was observed for serum creatinine concentration ($P = 0.005$). Serum concentrations of creatinine increased quadratically ($P < 0.001$) when betaine was supplemented during thermo-neutral conditions, showing the highest value at 0.10% of betaine. In contrast, serum concentrations of creatinine tended to decrease quadratically ($P = 0.068$) when betaine was supplemented to pigs in the heat-stressed environment, showing the lowest value at 0.15% of betaine inclusion.

Serum concentration of P was reduced due to heat stress, and this decrease was more pronounced on d 28 (interaction, $P = 0.021$). In addition, supplementation of betaine linearly

reduced serum P concentration (P = 0.006) when pigs were housed under thermo-neutral conditions. Conversely, supplementation of betaine tended to quadratically decrease serum P concentration (P = 0.06) when pigs were housed under heat-stressed conditions, showing the lowest concentration at 0.10% betaine (interaction, P = 0.056). Supplementation of betaine reduced serum CPK activity quadratically (P = 0.002) only on d 3 of heat exposure, showing the lowest activity at 0.15% betaine. Serum activity of CPK in heat-stressed pigs on d 28 was similar to the CPK levels of pigs housed under thermo-neutral environment (interaction, P = 0.001).

Serum concentration of total protein (P = 0.017) and amylase (P = 0.063) were greater on d 28 compared to d 3, regardless of the environment. Serum glucose concentration was reduced on d 28 regardless of the environment (P = 0.002). Chloride was not affected by the environment, betaine supplementation or day of measurement ($P \geq 0.260$).

Complete Blood Count

A three-factor interaction was observed for hematocrit percentage (P = 0.024). Betaine supplementation increased hematocrit quadratically only for pigs housed in the heat-stressed environment on d 28 (quadratic effect, P = 0.029); showing the highest level at 0.10% betaine. Supplementation of betaine did not affect hematocrit in pigs housed under thermo-neutral conditions nor heat-stressed pigs on d 3 ($P \geq 0.292$).

Hemoglobin decreased due to heat-stress and this reduction was more pronounced on d 28 of heat exposure (interaction, P = 0.024). Monocytes decreased on d 28 when pigs were housed in the thermo-neutral room. Monocytes count of heat-stress pigs were similar to the monocytes count in pigs housed in the thermo-neutral room on d 3 (interaction, P = 0.082). In

addition, supplementation of betaine increased monocyte count in a quadratic manner (P = 0.036), showing the greatest count at 0.10% betaine, regardless of the environment or day.

Eosinophil count was increased on d 28 when pigs were housed under heat-stressed conditions; conversely, eosinophil count was reduced on d 28 when pigs were housed in the thermo-neutral room (interaction, P = 0.002). In addition, eosinophils increased quadratically with betaine supplementation (P < 0.001), and the highest count of eosinophils was observed when betaine was added at 0.10%, regardless of the environment or day of measurement.

WBC increased on d 3 as compared to d 28, regardless of the environment (P = 0.003). Supplementation of betaine increased WBC in a quadratic manner (P = 0.037), showing the greatest count at 0.10% betaine. Platelet count was reduced on d 28 as compared to d 3, regardless of the environment (P = 0.005). Supplementation of betaine linearly decreased platelet count (P = 0.043), regardless of the environment or day of measurement. Neutrophils count decreased on d 28 as compared to d 3, regardless of the environment (P = 0.011). Supplementation of betaine at 0.10% increased (P = 0.051) neutrophils count, regardless of the environment or environment. Red blood cell count was greater on d 28 regardless of the environment (P = 0.068).

Discussion

High environmental temperatures cause significant reductions in profitability in swine production (St-Pierre et al., 2003). Understanding the impact of heat stress on the physiology of the pig can help to evaluate potential dietary strategies to maintain pork production during the summer heat. In the present study, we evaluated the use of the anhydrous betaine in diets

of growing pigs at incremental levels to determine if the osmoprotectant capacity of betaine could contribute to overcoming the cascade of negative effects caused by heat stress.

Body weight is negatively correlated with heat tolerance (Renaudeau et al., 2011). During the course of the experimental period (d 0 to d 28), pig BW ranged from 45.3 to 66.7 kg. Temperatures created in the heat stress environment were considerably higher than the upper critical temperature of the thermo-neutral zone suggested for this range of BW, which is approximate between 22.5 and 24.0°C (Renaudeau et al., 2011). Negative consequences of heat stress were clearly observed, as evidenced by the increase in rectal temperature, respiration rate, and poor growth. The poor growth was mostly associated with a reduction in ADFI. Pigs decrease ADFI as a mechanism to reduce metabolic heat (Renaudeau et al., 2011) that is associated with the activity of eating and increased heat increment associated with digestion and metabolism (Nienaber et al., 1996). In our experiment, heat stress reduced ADFI and ADG by 21 and 14%, respectively; while G:F increased 7%. Renaudeau et al. (2011) estimated that pigs with a BW of 75 kg would reduce ADFI by 13% and ADG by 9% when temperatures reached up to 34°C. They further estimated that G:F remains constant up to 30°C and decreases at higher temperatures.

In the present study, betaine supplementation reduced rectal temperature when included at 0.10%, and linearly decreased respiration rate in heat-stressed pigs, suggesting that betaine supplementation helped to offset hyperthermia. Similar effects were observed in heat-stressed rabbits in which dietary betaine (0 to 0.10%) linearly reduced rectal temperature and respiration rate (Hassan et al., 2011). Betaine supplementation (0.05 to 0.15%) to heat-stressed poultry has been shown to increase ADG, ADFI, and G:F (Farooqi et al., 2005; Sayed and

Downing, 2011; He et al., 2015) and reduce mortality (Zulkifli et al., 2004; Awad et al., 2014). We observed that betaine supplementation did not affect ADG or ADFI, regardless of inclusion level or environmental conditions. However, betaine increased G:F when included at 0.10% in pigs housed in the thermo-neutral and heat-stressed environment. This increase in G:F appeared to be due to a numeric reduction in ADFI (-90 g/d) when pigs were fed 0.10% betaine in the thermo-neutral environment; however, the improvement in G:F in pigs fed 0.10% betaine and housed in the heat-stressed room was due to a numeric increase in ADG (48g/d). The effect of betaine supplementation under conventional housing (no heat-stress) on pig performance was reported in a meta-analysis conducted by Sales (2011). Betaine consistently reduced the amount of feed per unit of gain (by 0.361, 12 studies), without affecting ADG (13 studies). The effect on ADFI (11 studies) was variable, but numerically decreased ADFI (Sales, 2011).

Dietary supplementation of betaine to energy-restricted pigs improved growth (Schrama et al., 2003; Dunshea et al., 2009). This effect was associated with a reduction in energy requirements for maintenance (Schrama et al., 2003). Betaine is proposed to reduce the energy requirements for maintenance through improved osmotic regulation, which will increase energy availability for growth (Schrama et al., 2003). Heat-stressed pigs in the present study had lower energy intake because of reduced ADFI, but they did not respond to a greater extent due to betaine supplementation than pigs housed under thermo-neutral conditions.

During heat stress, blood flow increases in the periphery to dissipate metabolic heat. As a compensatory mechanism, blood flow decreases in the splanchnic organs limiting the supply of oxygen and nutrients. Consequently, shortage of oxygen impairs ATP regeneration (Cronjé, 2005). Depletion of ATP impairs the activity of Na^+/K^+ ATPase and Ca^+ ATPase,

which are key enzymes regulating ion balance. Moreover, heat stress causes greater water losses due to evaporative cooling, and Na^+ concentrations rise in the extracellular compartment, causing a hypertonic stress (Houpt, 2004). The osmotic pressure is such that water and Na^+ enter the cell, causing cell swelling and rupture of the cell membranes (Cronjé, 2005). Disruption of the cell membrane allows an unregulated Na^+ and Ca^+ influx and K^+ efflux, resulting in increased ion ATPase activity (Cronjé, 2005).

In the present study, pigs exposed to heat stress had lower serum concentrations of Na^+ and higher concentrations of K^+, especially on d 3 of heat exposure, which may be related to disruption of the cell membrane. Pigs were able to maintain Na^+ concentrations within normal ranges (135 to 150 mEq/L) (Carr, 1998) despite the effect of heat stress. Levels of K^+ were above the normal range (6.7 mEq/L) (Carr, 1998) at the beginning of heat stress (d 3), with the exception of pigs fed 0.15% betaine. Nonetheless, exposure to prolonged heat amended Na^+ and K^+ serum concentration, because values were comparable with the thermo-neutral counterparts on d 28. Measurements of rectal temperature and respiration rate suggested that the peak of heat stress occurred at d 1. Possibly, a greater ion disturbance could have been observed on d 1. Pearce et al. (2013a) reported an increase in activity of Na^+/K^- ATPase pump in the intestinal tract on d 1 of heat stress, and no effect on d 3 and 7.

Elevated values of osmolarity indicate dehydration. In the present study, osmolarity values were considered normal (300 to 315 mOsm/L) (Harpur and Popkin, 1965; Waymouth, 1970) regardless of the environment, suggesting that pigs did not experience dehydration. Early exposure to heat stress (d 3) reduced osmolarity, while prolonged periods of heat stress (d 28) showed osmolarity values to be equal to pigs housed in the thermo-neutral room.

Patience et al. (2005) also reported lower serum osmolarity in heat-stressed (38 °C) pigs as compared to pigs housed in a thermo-neutral environment (20 °C). They related this effect to greater water intake or loss of electrolytes in the urine. Although we did not measure individual water consumption, overall water disappearance of the pigs housed in the heat-stress room was 3 folds greater than that of pigs housed in the thermo-neutral room.

In the present study, osmolarity of heat-stressed pigs was reduced due to betaine inclusion at 0.10%. Supplementation of betaine at 0, 0.05, and 0.10% in the water of heat-stressed (32°C) broilers was shown to increase water retention at 0.05%, but it did not affect osmolarity (Sayed and Downing, 2011). Greater water retention in heat-stressed broilers is considered to be beneficial in improving evaporative cooling and help reduce hyperthermia (Smith and Teeter, 1989). The quadratic response of betaine supplementation on osmolarity and rectal temperature may be related to improved hydration allowing the pig to enhance evaporative cooling when pigs were fed 0.10% betaine.

Multiple organ failures have been observed in the rodent model (Lim et al., 2007) and individuals with heat stroke (Bouchama and Knochel, 2002). For instance, heat stroke patients (rectal temperature of about 41 to 43°C) had increased concentrations of plasma ALT by 411%, AST by 590%, and CPK by 89% (Alzeer et al., 1997). Their relative increase in the plasma reflects the magnitude of tissue damage due to thermal injury (Alzeer et al., 1997). High levels of AST and ALT are indicators of liver injury and it is generally considered to be biological evidence of liver damage when there is a threefold increase in plasma concentrations beyond the upper range (Whalan, 2015). In addition, increased levels of plasma CPK indicate cardiac muscle, skeletal muscle, and brain injury (Whalan, 2015). On d 3, serum AST increased 32%

in pigs housed in the heat-stressed environment compared to pigs housed in the thermo-neutral room, while ALT was reduced by 2% in heat-stressed pigs. This suggests that there was minimal liver damage due to heat stress. There are few studies that have determined markers of tissues injury in heat-stressed pigs (Hicks et al., 1998; Pearce et al., 2013b) and none of them reported changes in plasma ALT, AST or CPK. In broilers, exposure to heat stress (32 °C) increased plasma CPK activity by 54% (Sandercock et al., 2001). In the present study, heat stressed pigs showed a marked increase in the activity of CPK on d 3 of heat exposure, in particular in pigs fed no supplemental betaine (by 853%). Heat-stressed pigs fed 0.15% betaine had the lowest increase in CPK activity (increase was 58%) as compared to pigs housed in the thermo-neutral room. These findings suggest that betaine may reduce cardiac and skeletal muscle damage due to heat stresss, uniquely during the first days of heat exposure because CPK activity on d 28 did not differ from pigs housed under thermo-neutral conditions. The physiological and anatomical adaptations that pigs experience during heat stress are not yet fully understood. However, reduced heart size (Heath, 1989; Nienaber et al., 1996; Cruzen et al., 2014) and increased liver size (Li et al., 2015) have been reported in heat-stressed pigs.

Increased serum creatinine and urea N concentrations are indicators of kidney malfunction (Whalan, 2015). However, urea N can be affected by protein intake while creatinine is not. In the present experiment, heat-stressed pigs had reduced urea N on d 3 by 17%, but no effect was observed on d 28 compared to pigs in the thermo-neutral environment. Clearly, the reduced feed intake due to heat stress is expected to decrease urea N concentrations and, thereforeurea N would not be a reliable indicator to determine kidney malfunction in ad libitum fed pigs. Heat-stressed pigs had a 20% increase in serum creatinine concentration

compare to pigs housed in the thermo-neutral environment. Greater accumulation (50%) of creatinine has been reported in heat-stressed pigs (Pearce et al., 2013b). The increase in creatinine indicates challenging conditions for the kidneys during heat-stress, but concentrations were still within normal ranges (0.8 to 1.7 mg/dl) (Carr, 1998).

Greater accumulation of adipose tissue and reduced lean deposition have been extensively observed in pigs reared under heat stressed conditions (Verstegen et al., 1973; Stahly et al., 1979; Bridges et al., 1998), and this effect has also been observed in broilers (Geraert et al., 1996). Pearce et al. (2013b) reported that heat-stressed pig had greater insulin levels, which shifts metabolism towards fat synthesis. This is further supported by reduced serum concentrations of NEFA, an intermediate of fatty acid oxidation, in heat-stress pigs (Rhoads et al., 2013; Sanz Fernandez et al., 2015). We observed reduced levels of NEFA and increased levels of triglycerides in pig exposed to heat, which is consistent with previous observations of increased fat deposition.

Hematology analysis did not reveal major differences due to heat stress. Hicks et al., (1998) evaluated hematology indices in pigs (4 wk-old) exposed to heat stress, but did not report changes. Supplementation of betaine had a quadratic effect on hematocrit, WBC, monocytes, and eosinophils, regardless of the environment, showing the greatest levels at 0.10% betaine. All values were considered in the normal range for growing pigs (Carr, 1998).

Heat stress markedly reduced pig growth performance. Respiration rate and rectal temperature of heat-stressed pigs gradually adapted to high environmental temperatures. Heat stress disrupted serum Na^+ and K^+ balance, especially in the first days of heat stress. Heat-stressed pigs did not demonstrate major organ injury, with the possible exception of cardiac

and skeletal muscle during the acute phase of heat stress. Supplementation of betaine alleviated heat stress by improving feed efficiency and reducing rectal temperature. The osmoprotectant capacity of betaine was supported by amending ion balance and preventing cardiac and skeletal muscle injury during the initial days of heat exposure. Therefore, supplementation of betaine had a minor impact on alleviating heat stress with the possible exception of early days of heat exposure.

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Table 1. Composition of experimental diets, as fed basis[1]

	Betaine Inclusion, %			
Item	0	0.10	0.15	0.20
Ingredient, %				
Corn, yellow dent	56.38	56.33	56.30	56.27
Corn dried distillers grains with solubles	20.05	20.03	20.02	20.01
Soybean meal, 47.5% CP	17.81	17.79	17.78	17.77
Poultry fat	2.51	2.50	2.50	2.50
Limestone	1.46	1.46	1.46	1.46
Monocalcium phosphate, 21% P	0.53	0.53	0.53	0.53
Salt	0.50	0.50	0.50	0.50
L-Lys·HCl	0.42	0.42	0.42	0.42
Trace mineral premix[2]	0.15	0.15	0.15	0.15
L-Thr	0.10	0.10	0.10	0.10
DL-Met	0.05	0.05	0.05	0.05
Vitamin premix[3]	0.03	0.03	0.03	0.03
Phytase[4]	0.01	0.01	0.01	0.01
Betaine[5]	0.00	0.10	0.15	0.20
Calculated ME Mcal/kg	3.41	3.41	3.41	3.41
SID Lys g:ME Mcal	2.96	2.96	2.96	2.96
Analyzed composition, as-fed basis				
Crude Protein, %[6]	19.17	19.55	19.72	19.53
Lys, %[6]	1.105	1.134	1.114	1.115
Gly, %[6]	0.756	0.777	0.762	0.745
Met, %[6]	0.353	0.352	0.347	0.345
Betaine,%[7]	0.13	0.29	0.36	0.36
Crude Fat, %[8]	6.22	6.06	6.06	6.17
Crude Fiber, %[8]	8.79	7.97	8.07	8.16
Ca, %[8]	0.64	0.73	0.7	0.68
P, %[8]	0.54	0.53	0.52	0.53

[1]Diets were formulated to meet or exceed NRC (2012).
[2]Supplied per kg of complete diet: 16.5 mg Cu as $CuSO_4$, 0.30 mg I as ethylenediamine dihydriodide, 165 mg Fe as $FeSO_4$, 40 mg Mn as $MnSO_4$, 0.30 mg Se as Na_2SeO_3, and 165 mg Zn as ZnO.
[3]Supplied per kg of complete diet: 8,227 IU of vitamin A, 1,172 IU of vitamin D_3 as D-activated animal sterol, 47.0 IU of vitamin E, 0.03 mg of vitamin B_{12}, 5.8 mg of riboflavin, 35.2 mg of niacin, 23.5 mg of d-pantothenic acid as calcium pantothenate, 3.8 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 1.7 mg of folic acid, 0.23 mg of d-biotin.
[4]Anhydrous betaine (Betafin®, Danisco A/S, Marlborough, Wiltshire, United Kingdom).
[5]Phytase (Optiphos ®, Huvepharm, Sofia, Bularia) (Phytase activity 2,000 U/g).
[6]Analyzed by Ajinomoto Heartland, Inc., Chicago, IL.
[7]Analyzed by Eurofins Scientific Inc., Des Moines, IA.
[8]Analyzed by the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO.

Table 2. Effects of ambient temperature and dietary betaine on growth pig performance[1].

	Enviroment[2]										
	Thermo-neutral				Heat-stressed				SEM	P - value	
	Betaine inclusion, %										
Item	0	0.1	0.15	0.2	0	0.1	0.15	0.2		Environment	Betaine
Body weight, kg											
d -7[3]	38.8	38.8	38.7	39.0	38.5	38.7	39.6	39.9	1.487	0.860	0.443
d 0	45.9	44.5	44.7	46.0	44.2	45.0	46.1	45.8	1.757	0.992	0.654
d 14	57.4	56.1	56.9	58.5	54.5	55.5	55.7	56.6	2.119	0.516	0.568
d 28	69.1	67.1	68.0	68.9	63.3	65.5	65.6	66.2	2.398	0.282	0.810
ADG, kg											
d -7 to 0[3]	1.015	0.820	0.865	0.994	0.820	0.885	0.911	0.848	0.133	0.642	0.933
d 1 to 14	0.883	0.824	0.871	0.895	0.739	0.746	0.696	0.773	0.047	0.004	0.623
d 15 to 28	0.840	0.787	0.791	0.743	0.625	0.712	0.711	0.682	0.043	0.020	0.678
d 1 to 28	0.832	0.806	0.831	0.819	0.682	0.730	0.702	0.728	0.039	0.008	0.971
ADFI, kg (as-fed basis)											
d -7 to 0[3]	1.77	1.75	1.77	1.78	1.83	1.69	1.73	1.75	0.106	0.896	0.848
d 1 to 14	2.29	2.13	2.23	2.41	1.73	1.68	1.69	1.78	0.135	0.001	0.403
d 15 to 28	2.33	2.24	2.16	2.31	1.83	1.92	1.92	1.92	0.122	0.009	0.908
d 1 to 28	2.28	2.19	2.28	2.36	1.78	1.80	1.81	1.85	0.131	0.002	0.783
G:F											
d -7 to 0[3]	0.559	0.480	0.484	0.545	0.451	0.516	0.501	0.487	0.065	0.639	0.980
d 1 to 14	0.393	0.389	0.391	0.375	0.429	0.451	0.410	0.442	0.016	0.014	0.580
d 15 to 28	0.366	0.355	0.348	0.327	0.343	0.375	0.374	0.358	0.021	0.396	0.701
d 1 to 28[4]	0.369	0.372	0.368	0.351	0.385	0.411	0.390	0.380	0.013	0.036	0.259

[1]Values represent least squares means of 8 pigs.
[2]The temperature curves were set for the following times, 0000, 0200, 0400, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200. Temperature set points for thermo-neutral conditions consisted in 15, 14, 15, 15, 17, 18, 19, 20, 21, 20, 17, and 15°C, for each time respectively. For heat-stressed conditions consisted in 29, 28, 29, 29, 31, 32, 33, 34, 35, 34, 31, and 29°C, for each time respectively.
[3]Dietary treatments were provided on d -7, environmental treatments started on d 0.
[4]Quadratic effect of betaine level (P = 0.071).

Table 3. Effects of dietary supplementation of betaine on serum chemistry of pigs housed under thermo-neutral and heat-stressed conditions at d 3 and 28 of ambient temperature exposure[1].

		Environment									
		Thermo-neutral				Heat-stressed					
		Betaine inclusion, %									
Item[2]	Day	0	0.10	0.15	0.20	0	0.10	0.15	0.20	SEM	Probability[3, 4]
Total Protein, g/dL	3	5.86	5.84	5.98	5.99	5.90	5.99	5.90	5.91	0.102	D*
	28	6.05	5.89	5.98	6.03	6.14	6.08	6.13	5.98		
Albumin, g/dL	3	3.913	3.863	3.863	3.850	3.863	3.740	3.749	3.875	0.066	B*, D***, L†, Q**
	28	4.118	3.975	4.000	4.075	4.025	3.854	3.890	3.888		
Globulin,g/dL	3	1.975	1.975	2.113	2.138	2.038	2.254	2.197	2.038	0.096	E*
	28	1.963	1.900	1.975	1.950	2.113	2.225	2.240	2.088		
Albumin/globulin ratio	3	1.90	1.99	1.86	1.86	1.91	1.70	1.60	1.94	0.109	E*, D*, Q†
	28	2.15	2.13	2.06	2.15	1.96	1.75	1.76	1.90		
P, mg/dl	3	9.35	9.00	8.90	8.90	8.75	8.29	8.37	8.61	0.199	E*, E×B†, D***, E×D*
	28	8.39	8.38	8.04	8.00	8.04	7.79	8.30	8.29		
Ca, mg/dl	3	10.88	10.70	10.88	10.88	10.60	10.48	10.33	10.61	0.106	E**, D***, Q†
	28	10.55	10.60	10.61	10.54	10.38	10.29	10.27	10.48		
Mg, mEq/L	3	1.74	1.66	1.66	1.69	1.68	1.65	1.66	1.70	0.151	E**, D***, E×D***
	28	2.08	1.96	1.93	2.09	2.76	2.64	2.70	2.73		
K, mEq/L	3	6.06	5.68	5.63	5.53	7.11	6.88	6.31	6.85	0.215	E*, D**, B×D*
	28	5.45	5.66	5.79	5.50	6.29	6.21	6.38	6.53		
Na, mEq/L	3	142.88	142.25	142.25	141.50	138.75	138.49	138.54	138.87	0.574	E*, D***, E×D**
	28	143.25	143.63	143.13	143.50	141.25	140.92	141.69	142.63		
Na/K Ratio	3	24.00	25.50	25.50	25.86	19.75	20.36	22.54	20.38	0.840	E***, D**, B×D†
	28	26.38	25.63	24.75	26.13	22.63	22.64	22.54	22.00		
Cl, mEq/L	3	102.00	101.75	101.88	101.38	102.00	101.84	102.47	102.00	0.652	
	28	100.63	101.38	102.00	101.25	101.50	102.84	102.18	102.25		
Urea N, mg/dl	3	16.8	14.9	14.9	15.3	13.3	12.0	12.6	13.3	1.098	E×D**, Q†
	28	15.3	13.1	13.6	13.6	14.1	13.3	14.2	14.0		
Creatinine, mg/dl	3	0.748	0.849	0.774	0.731	0.923	0.911	0.869	0.927	0.035	E***, E×B**, D***
	28	0.877	0.936	0.911	0.908	1.110	1.080	1.070	1.208		
Urea N/creatinine ratio	3	21.3	17.8	19.4	19.7	14.4	13.0	14.7	14.8	1.387	E***, D***, E×D*
	28	16.5	14.1	15.0	15.3	12.8	12.6	13.2	11.6		
Osmolarity, mOsm/L	3	309.5	307.2	306.9	305.2	302.6	300.0	300.1	302.0	1.291	E***, D***, E×D**, E×B†
	28	308.2	308.6	307.9	308.2	305.5	304.4	306.9	308.9		

[1]Values represent least squares means of 8 pigs.
[2]Phosphorous (P), calcium (Ca), magnesium (Mg), Potassium (K), Sodium (Na), Sodium potassium ratio (Na/K), Chloride (Cl), urea nitrogen (N).
[3]Effect abbreviations: E = environment, B = betaine, E×B = environment and betaine interaction, D = day, E×D = environment and day interaction, B×D = betaine and day interaction, E×B×D = environment, betaine, and day interaction, L = linear effect of betaine, Q = quadratic effect of betaine.
[4]Significance levels: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; †$0.10 > P > 0.05$.

Table 3 continued

		Environment									
		Thermo-neutral				Heat-stressed					
		Betaine inclusion, %									
Item[2]	Day	0	0.10	0.15	0.20	0	0.10	0.15	0.20	SEM	Probability[2, 3]
AST, U/L	3	27.5	29.5	27.5	32.3	43.3	38.0	37.7	35.9	3.849	E*, D**
	28	22.8	23.1	34.3	28.3	26.8	30.3	28.4	28.1		
ALT, U/L	3	27.5	26.3	28.5	28.3	29.1	27.9	24.8	26.4	2.476	E*, E×D*
	28	32.1	26.4	31.4	35.4	26.4	24.4	24.7	22.8		
AP, U/L	3	165	187	170	176	146	133	130	173	10.02	E***, B*, E×B*, D***, L*, Q†
	28	152	157	160	158	123	106	121	145		
GGTP, U/L	3	15.3	11.6	10.5	11.1	21.8	26.0	22.4	25.8	6.276	E**, D***, E×D*
	28	37.0	33.7	33.0	36.7	44.3	46.2	48.4	43.6		
Amylase, U/L	3	1523	1563	1364	1594	1388	1360	1444	1458	152.5	D†
	28	1607	1500	1411	1630	1636	1520	1789	1642		
Lipase, U/L	3	2.28	2.07	2.29	2.81	1.49	1.43	1.35	1.63	0.396	E***, D**, L†
	28	2.58	2.71	3.00	2.91	1.71	2.25	2.38	2.77		
CPK, U/L	3	2,277	3,622	2,392	2,474	25,655	8,131	4,255	21,057	1710	E***, E×B*, D***,
	28	1,428	1,115	2,187	1,705	1,607	1,669	1,631	3,055		E×D***, Q*
Cholesterol, mEq/L	3	109.3	106.6	104.8	105.8	106.4	102.7	114.0	116.4	4.587	E×B†, D**
	28	102.1	104.1	99.9	98.5	101.6	100.2	107.1	106.9		
Triglycerides, mEq/L	3	34.9	32.3	27.8	30.8	36.4	33.4	33.0	34.6	3.546	E*, D*
	28	29.8	29.4	24.3	23.5	32.6	32.8	29.3	40.6		
NEFA, mEq/L	3	0.081	0.087	0.098	0.091	0.078	0.081	0.082	0.092	0.010	E***, E×D***, B×D†
	28	0.137	0.110	0.102	0.113	0.077	0.069	0.062	0.059		
Glucose, mg/dl	3	100.1	107.8	104.2	98.2	109.4	103.9	103.4	104.9	4.435	D*
	28	96.2	96.0	93.3	96.2	95.4	97.9	99.6	99.6		

[1]Values represent least squares means of 8 pigs.
[2]Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ-glutamyltranspeptidase (GGTP), and creatine phosphokinase (CPK).
[3]Effect abbreviations: E = environment, B = betaine, E×B = environment and betaine interaction, D = day, E×D = environment and day interaction, B×D = betaine and day interaction, E×B×D = environment, betaine, and day interaction, L = linear effect, Q = quadratic effect.
[4]Significance levels: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; †$0.10 > P > 0.05$.

Table 4. Effects of dietary supplementation of betaine on serum concentrations of methionine, cysteine, and homocysteine in pigs housed under thermo-neutral and heat-stressed conditions at d 3 and 28 of ambient temperature exposure

		Environment									
		Thermo-neutral				Heat-stressed					
		Betaine inclusion, %									
Item	Day	0	0.10	0.15	0.20	0	0.10	0.15	0.20	SEM	Probability[2, 3]
Cysteine, µg/ml	3	141.9	119.8	146.3	141.3	162.8	145.6	142.0	162.1	16.42	E*
	28	105.5	111.8	125.2	124.4	159.0	146.9	136.1	161.7		
Methionine, µg/ml	3	9.40	9.57	9.37	9.25	7.53	7.61	6.52	7.75	0.6858	D**, E×D***
	28	7.04	6.63	7.07	6.27	7.67	8.13	7.48	8.56		
Homocysteine,	3	0.016	0.012	0.014	0.014	0.005	0.002	0.003	0.006	0.0047	E×D*
µg/ml	28	0.009	0.010	0.008	0.004	0.011	0.005	0.011	0.004		

[1]Values represent least squares means of 8 pigs.
[2]Effect abbreviations: E = environment, E×B = environment and betaine interaction, D = day, E×D = environment and day interaction.
[3]Significance levels: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$.

Table 5. Effects of dietary supplementation of betaine on complete blood counts in pigs housed under thermo-neutral and heat-stressed conditions at d 3 and 28 of ambient temperature exposure

		Environment									
		Thermo-neutral				Heat-stressed					
		Betaine inclusion, %									
Item[2]	Day	0	0.10	0.15	0.20	0	0.10	0.15	0.20	SEM	Probability[3,4]
WBC, 10^3/µL	3	15.5	17.3	15.3	16.0	14.2	17.6	15.5	15.3	1.045	B*, D**, Q*
	28	15.5	14.6	13.8	14.3	13.8	15.8	14.2	13.1		
RBC, 10^6/µL	3	7.62	7.94	7.85	7.69	7.99	7.59	7.80	7.78	0.211	D†
	28	7.96	8.11	8.14	8.15	8.00	8.00	7.70	7.61		
Hemoglobin, g/dL	3	13.11	13.69	13.19	13.11	13.86	13.19	13.68	13.25	0.340	E×D*
	28	13.79	13.61	13.99	13.55	13.40	13.58	13.25	12.88		
Hematocrit, %	3	41.4	43.3	42.0	41.4	42.7	41.5	43.4	42.3	1.129	E×B×D*, Q*
	28	42.6	43.1	43.3	43.3	42.3	44.8	41.5	40.0		
Platelet count, 10^3/µL	3	419	390	385	362	377	320	387	338	37.32	B*, D**, L*
	28	358	306	348	322	377	255	341	262		
Neutrophils, 10^3/µL	3	5.18	6.49	5.36	5.34	4.50	5.69	4.20	5.46	0.524	B†, D*
	28	4.23	5.60	4.52	5.25	4.94	4.31	4.09	4.14		
Lymphocytes, 10^3/µL	3	8.73	9.00	8.55	9.33	8.87	10.34	9.90	8.54	0.797	E×B*
	28	9.99	7.88	8.41	7.83	7.69	9.57	8.60	7.73		
Neutrophils/Lymphocytes	3	0.593	0.722	0.626	0.583	0.507	0.547	0.424	0.640	0.079	E×B†, E×D×B†
	28	0.423	0.710	0.537	0.671	0.618	0.450	0.475	0.535		
Monocytes/µL	3	791	787	765	689	504	798	722	622	81.08	B†, D*, E×D†, Q*
	28	691	720	411	596	657	734	662	538		
Eosinophils/µL	3	361	497	451	380	266	450	347	264	72.69	B**, E×D**, Q***
	28	330	388	338	220	392	644	437	329		
Basophils/µL	3	172	195	161	177	116	138	158	160	18.54	E×D†
	28	185	138	122	146	155	161	148	134		

[1]Values represent least squares means of 8 pigs.
[2]White blood cells (WBC), and red blood cells (RBC).
[3]Effect abbreviations: E = environment, B = betaine, E×B = environment and betaine interaction, D = day, E×D = environment and day interaction, B×D = betaine and day interaction, E×B×D = environment, betaine, and day interaction, L = linear effect, Q = quadratic effect.
[4]Significance level: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; †$0.1 > P > 0.05$.

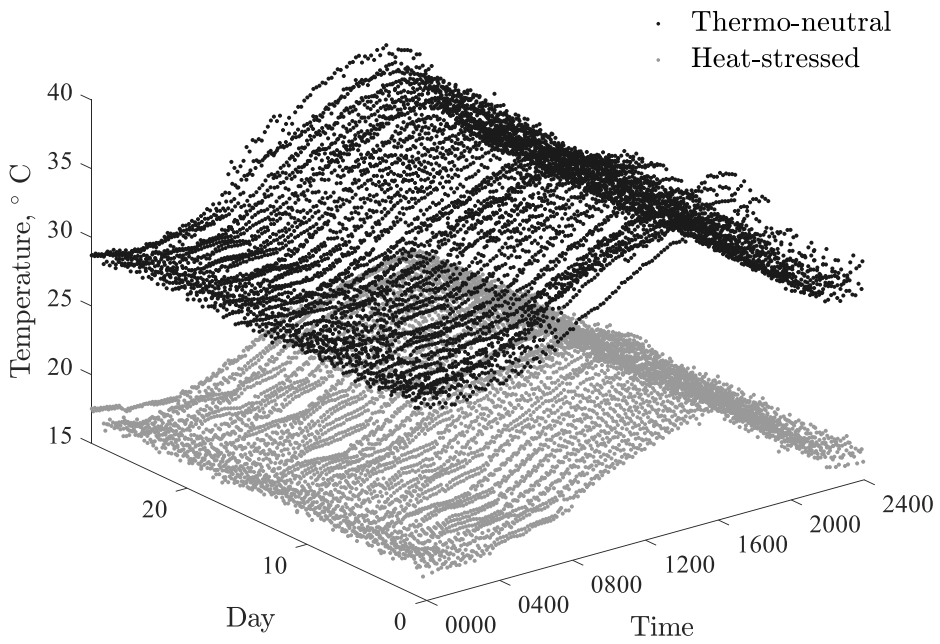

Figure 1. Room temperatures of the thermo-neutral and heat-stressed environments from d 0 to 28 in intervals of 10 min. Symbols represent individual temperature measurements by the temperature data recorders. During d 0 to d 28 target temperatures for the thermo-neutral environment were 15, 14, 15, 15, 17, 18, 19, 20, 21, 20, 17, and 15°C, for 0000, 0200, 0400, 0600, 0800, 1000, 1200, 1400, 16000, 1800, 2000, and 2200 h, respectively. During d 0 to d 28 target temperatures for the heat-stressed environment were 29, 28, 29, 29, 31, 32, 33, 34, 35, 34, 31, and 29°C for 0000, 02000, 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200 h, respectively. The overall average temperatures during d 0 to d 28 were 19.3 °C ±2.2 and 31.0°C ±3.1 for the thermo-neutral and heat-stressed rooms, respectively.

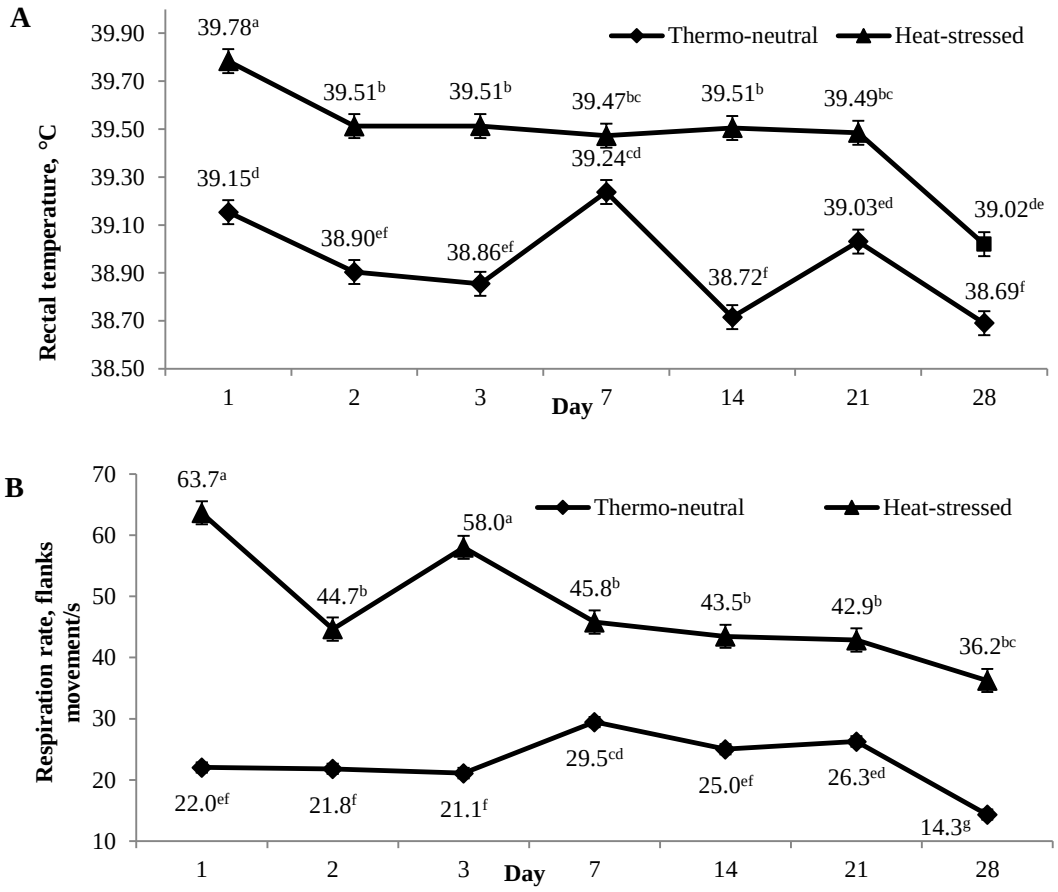

Figure 2. Effect of environmental temperature on rectal temperature and respiration rate on d 1, 2, 3, 7, 14, 21, and 28. Environment and day interaction ($P \leq 0.001$). Measurements were taken between 1400 and 1700 h. Symbols represent least squares means ± SEM of 32 pigs. Means with different superscript differ (a-g) ($P \leq 0.05$). **A.** Rectal temperature of heat-stressed pigs on d 1 was greater compared to rectal temperature of heat-stressed pigs on d 2 through 28. Rectal temperature of heat-stressed pigs on d 28 was similar to the rectal temperature of pigs housed in the thermo-neutral environment on d 1, 7, and 21. Rectal temperature decreased linearly ($P \leq 0.001$) across the days, regardless of the environment. **B**. Respiration rate was measured as the number of flank movements per 30 seconds. Respiration rate were higher on d 1 and 3 of heat exposure compare to measurements recorded in heat-stressed pigs on d 2, 7, 14, 21, and 28. Respiration rate measured on d 28 of heat stress was similar to the respiration rate of pigs housed in the thermo-neutral room on d 7. Respiration rate linearly decreased ($P \leq$ 0.001) across the days regardless of the environment.

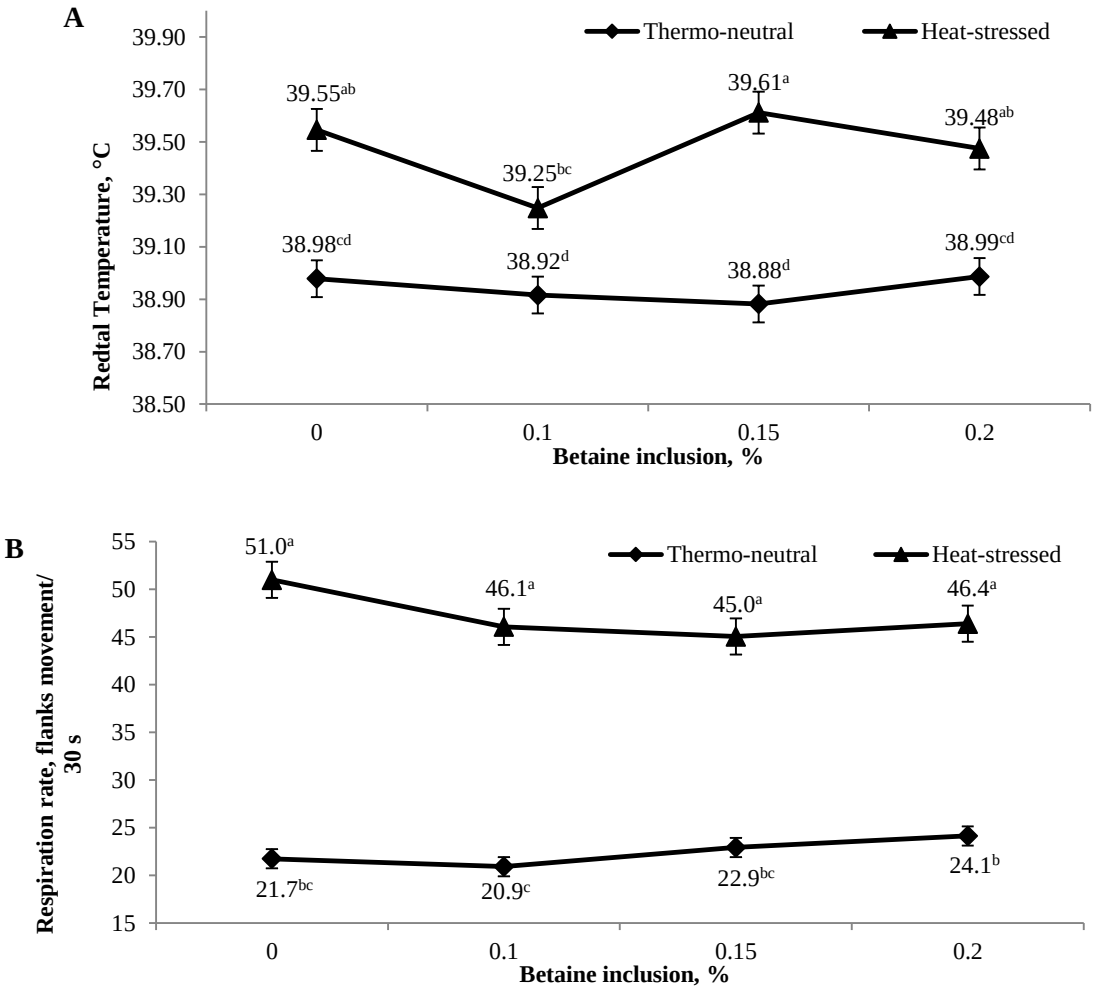

Figure 3. Effect of environmental temperature and betaine on rectal temperature and respiration rate. Rectal temperature and respiration rate were measured between 1400 and 1700 h and presented as the mean of measurements taken on d 1, 2, 3, 7, 14, 21, and 28. Symbols represent least squares means ± SEM of eight pigs and seven measurements in time (56 observation). Means with different superscript differ (a-d) ($P \leq 0.05$) **A.** An interactive effect of environmental temperature and betaine ($P = 0.055$) was observed. Betaine supplementation tended to reduce rectal temperature in a quadratic manner ($P = 0.071$) in pigs housed in the thermo-neutral environment, whereas betaine supplementation at 0.10% reduced ($P = 0.078$) rectal temperature in pigs housed in the heat-stressed environment. **B**. Respiration rate was measured as the number of flank movements per 30 seconds. Interactive effect of environment and betaine ($P = 0.040$). Betaine increased respiration rate linearly ($P = 0.037$) to pigs housed under thermo-neutral environment and linearly reduced ($P = 0.067$) respiration rate to pigs housed under heat-stressed environment.

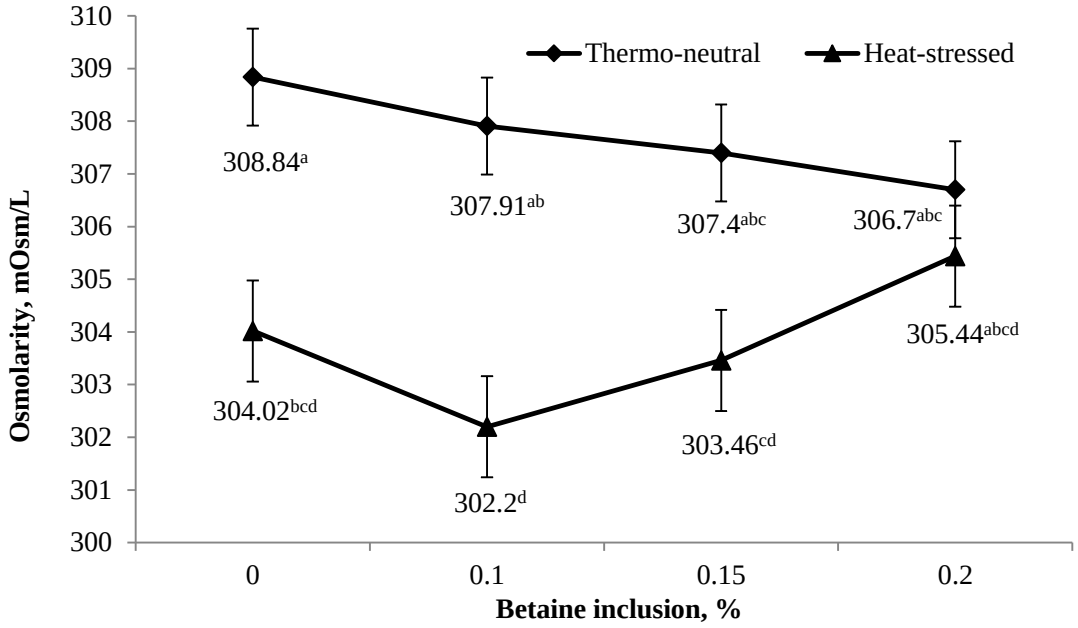

Figure 4. Effect of environment and betaine on serum osmolarity. Symbols represent least squares means ± SEM of 8 pigs and 2 measurements (d 3 and d 28) in time (16 observations). Means with different superscript differed (a-d) ($P \leq 0.05$). A tendency for an interactive effect of environment and betaine ($P = 0.075$) was observed. Betaine reduced osmolarity quadratically ($P = 0.013$) in pigs housed in the heat-stressed environment, but linearly decreased osmolarity ($P = 0.10$) in pigs housed in the thermo-neutral environment.

CHAPTER III:

Effect of dietary betaine and ractopamine HCl on growth and carcass characteristics in finishing pigs housed under high ambient temperatures

Abstract: Betaine is an osmolyte that helps to maintain water homeostasis and cell integrity, which is essential during heat stress. We hypothesized that supplemental betaine improves growth during heat stress and that this effect may be greater in pigs fed ractopamine. Two studies were conducted to determine: 1) the effects of betaine in combination with ractopamine; and 2) the optimal betaine level for late finishing pigs. High environmental temperatures were imposed by gradually increasing temperatures over 10 d to the target high temperature of 32°C. In Exp. 1, pigs (n = 1,477, BW = 91.6 ± 3 kg) were assigned within weight blocks and sex to 1 of 4 dietary treatments arranged in a 2 x 2 factorial RCB design. Treatments consisted of diets without or with ractopamine (5 ppm for 21 d followed by 8.8 ppm to market) and each of these diets supplemented with either 0 or 0.2% of betaine. Pigs were housed 20 to 23 pigs per pen using a total of 68 pens. Betaine reduced ($P \leq 0.05$) BW (123.1 vs. 124.3 kg), ADG (0.780 vs. 0.833 kg/d), and ADFI (2.800 vs. 2.918 kg/d), but did not impact carcass characteristics. Ractopamine increased ($P < 0.01$) BW (125.53 vs. 121.87 kg), ADG (0.833 vs. 0.769 kg/d), and G:F (0.295 vs. 0.265) and reduced ADFI (2.822 vs. 2.896 kg/d, $P = 0.033$). Supplementation with ractopamine increased ($P < 0.001$) HCW (94.11 vs. 90.03 kg), carcass yield (74.78 vs. 73.76%), loin depth (63.56 vs. 59.98 mm), and lean percentage (53.18 vs. 51.69%), and reduced backfat depth ($P < 0.001$; 20.21 vs. 22.45 mm). In Exp. 2, pigs (n = 2,193, BW = 95.5 ± 3.5 kg) were assigned within weight blocks and sex to 1 of 5 dietary treatments in a RCB design. Pigs were housed in pens with 20 to 24 pigs each, using a total of 100 pens. Treatments consisted of diets supplemented with 0, 0.0625, 0.125, 0.1875% of betaine, and a positive control diet supplemented with ractopamine as in Exp. 1. Betaine decreased carcass yield in a quadratic manner ($P = 0.076$; 74.09, 73.54, 73.78, and

73.89 for 0, 0.0625, 0.125, 0.1875% of betaine, respectively), but did not impact other responses. Ractopamine improved ($P < 0.001$) G:F (0.334 vs. 0.295), market weight (121.6 vs. 118.5 kg), carcass yield (74.7 vs. 73.8%), loin depth (61.68 vs. 58.99 mm), and lean percentage (53.20 vs. 52.58%), and reduced backfat (18.78 vs. 20.35 mm). Collectively, data indicate that under commercial conditions, betaine did not improve pig performance housed under high ambient temperatures, regardless of ractopamine inclusion. Ractopamine improved growth and carcass characteristics of pigs housed under high ambient temperatures.

Keywords: betaine, finishing pigs, heat stress, ractopamine.

Introduction

High ambient temperatures cause significant losses to the livestock industry (St-Pierre et al., 2003). Heat-stressed pigs have reduced growth and feed efficiency, increased mortality, reduced market weight, and decreased carcass value (Rhoads et al., 2013). Evaluating potential dietary strategies to minimize the negative effects of high ambient temperatures is of great interest.

Betaine or tri-methyl glycine is known for its methyl-donor and osmolyte properties (Kidd et al., 1997; Craig, 2004). Betaine is found at relatively high concentrations in plants that are adapted to drought, salinity, and extreme temperatures (Ashraf and Foolad, 2007). As an osmolyte, betaine was reported to reduce the activity of ion ATPase pump of the erythrocyte (Moeckel et al., 2002) and maintain ion balance. Betaine has been shown to reduce energy requirements for maintenance (Schrama et al., 2003), but effects on performance (Haydon et al., 1995; Matthews et al., 1998; Lawrence et al., 2002) and carcass characteristics (Cadogan et al., 1993; Yu et al., 2001; Rikard-Bell et al., 2009) have been variable. Interestingly, the

response to betaine appears to be more pronounced in pigs fed energy restricted diets (Schrama et al., 2003; Dunshea et al., 2009).

Ractopamine is a β-androgenic agonist that binds to the β-androgenic receptors found in skeletal muscles and adipose tissues (Yang and McElligott, 1989). Ractopamine causes muscle hypertrophy through increased protein synthesis (Bergen et al., 1989), thus promoting lean deposition. Maintenance energy requirements and heat production are positively correlated with body protein mass and greater in lean pigs (de Lange et al., 2001; Brown-Brandl et al., 2004).

It is hypothesized that betaine can be effectively used as a heat abatement strategy in finishing pigs and that this response is larger in pigs fed ractopamine. The objectives were to determine the optimal level of betaine and the effects of betaine in combination with ractopamine on performance of late finishing pigs.

Materials and Methods

Two experiments were conducted at the Finishing Research Farm of Hanor Company (Finish Site 727, White Hall, IL). Pigs (TR-4 x Camborough product sows, Pig Improvement Company, Hendersonville, TN) were housed in curtain-sided finishing barns in pens (2.67 x 5.64 m^2) with concrete slatted floors. Pens had 4 nipple water drinkers and a 4-space feeder with a capacity of 109 kg. Pigs used in these experiments were treated humanely and procedures used were consistent with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). The experiment was conducted under the supervision of licensed veterinarians.

The effect of experimental diets on pig growth performance and carcass characteristics were evaluated under heat stressed conditions. Heat stress was gradually imposed and acclimation to high temperatures was initiated 10 days before dietary treatments were provided. Initial barn temperature was set at 21°C and then increased 1°C per day. Temperatures were controlled by using heaters, raising side curtains, and by reducing the rate of ventilation in the barn. Ventilation in the barn was maintained by the continuous operation of a minimum ventilation fan to ensure sufficient air flow to provide good air quality. The target high temperature was in the range of 31 to 32°C from 6:00 h to 22:00 h and the target low temperature was in the range of 26 to 27°C from 22:01 h to 5:59 h. Water misters were programmed to turn on at 33°C as a safety precaution to avoid excessive heat stress.

Experiment 1

Upon arrival into the finisher barns, barrows and gilts were housed in separate barns and placed in pens by weight. Pigs were fed a common 5-phase feeding program until they reached the target weight for the study (approximately 90 kg). The study was conducted from May to June for a period of 7 weeks. A total of 1,477 pigs were used, consisting of 727 barrows and 750 gilts (initial BW of 91.6 ± 3.0 kg). Pigs were housed 20 to 23 pigs per pen with 17 pens per treatment using a total of 68 pens. Pigs were weighed and pens were blocked by initial BW of pigs in the pen and randomly assigned within BW block to 1 of 4 dietary treatments (Table 1). Treatments were arranged in a 2 x 2 factorial randomized complete block design and consisted of diets without or with ractopamine (ractopamine HCl, Paylean® 9, Elanco Animal Health, Greenfield, IN) and each of these diets was supplemented with either 0 or 0.2% of natural betaine (Betafin®, Danisco A/S, Marlborough, Wiltshire, United Kingdom). Diets

were manufactured by a commercial feed mill (Hanor Company, Greenfield, IL). Ractopamine was included at 5.0 ppm for 21 d followed by 8.8 ppm until pigs reached market weight. Diets containing ractopamine had increased concentrations of amino acids, minerals and vitamins (Table 1) to account for increased muscle accretion associated with ractopamine supplementation (Schinckel et al., 2003).

Feed and water were provided *ad libitum* throughout the entire experiment. Experimental diets were provided using an automated feeding system (Howema, Big Dutchman, Vechta, Germany). The system dispensed the appropriate treatment diet to each pen and recorded the amount of feed delivered by weight. Feeders were checked daily to ensure feed was available to the pigs at all times and were adjusted appropriately to minimize feed wastage. Feed additions were determined on a daily basis. The accuracy of the feeding system was checked on a weekly basis by capturing and weighing feed delivered at 2 locations randomly distributed in each barn.

Experiment 2

Upon arrival to the finisher barns, barrows and gilts were housed in separate barns and placed in pens by weight. Pigs were fed a common 5-phase feeding program until they reached target weight for the study. The study was conducted from August to October for a period of 6 weeks. A total of 2,193 pigs were used, consisting of 1,121 barrows and 1,072 gilts (initial BW of 95.5 ± 3.5 kg). Pigs were housed 20 to 24 pigs per pen with 20 pens per treatment. Pens were blocked by initial BW of pigs in the pen and assigned randomly within block to 1 of 5 dietary treatments (Table 1). Treatments consisted of diets supplemented with 0, 0.0625, 0.125, or 0.1875% of betaine and an additional diet supplemented with ractopamine. Ractopamine

was included at 5 ppm for 21 d followed by 8.8 ppm until pigs reached market weight. Similar to Exp.1, diets containing ractopamine were supplemented with additional amino acids, minerals, and vitamins to account for the increased lean accretion associated with ractopamine. Diets with 0 or 0.1875% betaine and the ractopamine diet were manufactured by a commercial feed mill (Hanor Company, Greenfield, IL) and were color coded to ensure that the proper experimental diets were fed. Diets with intermediate concentrations of betaine were mixed by the Howema system using the 0 and 0.1875% betaine diets.

Feed and water were provided *ad libitum* throughout the entire experiment. Feeders were checked daily to ensure feed was available to the pigs at all times. Feed was added to each feeder by the Howema system and additions were recorded by weight on a daily basis. The accuracy of the feeding system was checked on a weekly basis by capturing and weighing feed delivered at 2 random locations in each barn.

Measurements

Proximate analysis of the experimental diets was conducted by the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC (2005) procedures. Diets were analyzed for CP (Method 990.03), crude fat (Method 920.39 (A)), crude fiber (Method 978.10), Ca (Method 985.01 (A, B, D)) and P (Method 966.01). The content of anhydrous betaine in the final diets was analyzed by Eurofins (Eurofins Scientific Inc., Des Moines, IA) using capillary electrophoresis.

In Exp. 1 ambient room temperatures were recorded daily by using high-low thermometers. One thermometer was placed in each experimental barn. Two cohort barns parallel to the experimental barns, which housed pigs similar in weight and age to the ones in

the current experiment, were used to measure ambient room temperatures. In Exp. 2 ambient room temperatures were measured by data loggers (LogTag, MicroDAQ Ltd., Contoocook, NH). Three devices were placed in each experimental barn at the approximate height of the pigs. Measurements were recorded every 10 min. Similar to Exp. 1, cohort barns were used to contrast ambient room temperatures with experimental barns.Feed samples were collected at the feed mill and from random sentinel feeders within the barn. Samples were analyzed to chemically verify treatments.

In both experiments, BW of pigs within each pen was measured on d 0, immediately prior to the first marketing group, and at each subsequent marketing group. Marketing of pigs was performed by visually identifying the heaviest pigs within pen. The selected pigs within each pen were weighed together and tattooed with their pen number 24 hours prior to being shipped to the packing plant (Triumph Foods Inc., St. Joseph, MO). This procedure was repeated 3 to 4 times in the course of 3 weeks until there were no remaining pigs in the pen. Each of the trucks was loaded with equal numbers of pigs per treatment. Feed intake of the pen was measured from the difference of daily feed additions and the weight of remaining feed each time pigs were weighed. Pig BW, ADG, ADFI, and G:F were calculated per pen, considering the number of pigs in each pen and days to market (sum of the number of days of each pig in the pen for the marketing group).

Hot carcass weight, backfat thickness, and loin depth were determined at the packing plant by identifying pen tattoo. Carcass yield was determined using the average live pig BW of the group of pigs in each pen that were marketed and the average HCW of these pigs for each pen. Backfat and loin depth were measured with an optical probe (SFK, Herlev, Denmark). The

probe was inserted into the carcass between the 3rd and 4th last rib at a distance of approximately 7 cm from the midline. Lean percentage was determined by the equation used at the packing plant as follows:

$$Lean, \% = \frac{2.827 + (1.0340\ x\ HCW, kg) - (0.7272\ x\ backfat, mm) + (\ 0.3868\ x\ loin\ depth, mm))}{2.2046\ x\ HCW, kg}$$

The weight of dead and removed pigs and the date of removal were recorded. Dead and removed pig weights were included in the total pen weight and the days they were on test were included in the total days for the marketing group to calculate performance data. Non-ambulatory pigs and dead pigs upon arrival at the packing plant were considered as missing observations for the data of carcass characteristics.

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was used as the experimental unit. In Exp. 1 the model tested for fixed effects of betaine, ractopamine, sex, and their interactions. Initial BW was used as a covariate, given that it differed among treatments. Weight block nested within sex was used as a random effect. In Exp. 2, the model tested for the fixed effects of diet treatment, sex, and their interaction. Weight block nested within sex was used as a random effect. Least squares means of the treatments were compared using Tukey's method. Orthogonal contrast comparisons were conducted to determine linear and quadratic effects of betaine level.

In both experiments, HCW was used as a covariate when significant ($P < 0.01$) to adjust carcass characteristics. Least squares means were reported, and differences were considered statistically significant at $P \leq 0.05$ and were considered tendencies when $0.05 < P \leq 0.10$.

Results

Experiment 1

Average lowest temperature for the experimental barns was 26 ± 1°C and average highest temperature were 31 ± 2°C, average lowest temperature for the experimental barns was 21 ± 1°C and average highest temperature were 30 ± 3°C (Figure 1). Analyzed dietary betaine concentration was 0.192% for the control diet with added betaine and 0.186% for the ractopamine diets with added betaine (Table 1). Betaine was not detectable in the diets without supplemental betaine.

No effect due to betaine ($P = 0.841$) or ractopamine ($P = 0.494$) were observed on pig mortality. Supplementation of betaine reduced ($P \leq 0.05$) BW (123.1 vs. 124.3 kg), ADG (0.780 vs. 0.833 kg/d), and ADFI (2.800 vs. 2.918 kg/d) (Table 2). An interaction ($P = 0.015$) of betaine and sex was observed for G:F ratio from d 0 to d 27 (data not shown). Supplementation of betaine reduced G:F for barrows (0.324 vs. 0.335), but not for gilts (0.330 vs. 0.326). No overall effects due to betaine were observed for G:F or days to market ($P \geq 0.198$).

Supplementation of ractopamine increased ($P < 0.01$) BW (125.53 vs. 121.87 kg), ADG (0.833 vs. 0.769 kg/d), and G:F (0.295 vs. 0.265). Ractopamine reduced ADFI from d 0 to market (2.822 vs. 2.896 kg/d, $P = 0.033$). No effect of ractopamine was observed for days to market ($P = 0.463$).

Barrows had greater BW (P = 0.02), ADG (P < 0.001), ADFI (P < 0.001), and they reached market earlier (P < 0.001) than gilts (Table 2). No differences in G:F were observed between barrows and gilts.

Betaine tended (P = 0.06) to reduce HCW by 0.82 kg; however, no other effects of betaine on carcass characteristics were observed (Table 3). Supplementation of ractopamine increased HCW, carcass yield, loin depth, and lean percentage by 4.1 kg, 1.03%, 3.57 mm, 1.49%, respectively, and reduced backfat depth by -2.25 mm (P < 0.001). Gilts had reduced back fat and increased loin depth and lean percentage as compared to barrows (P < 0.01). An interaction (P = 0.015) of the three factors was observed for carcass yield. Supplementation of betaine to diets containing ractopamine reduced carcass yield in barrows (74.47 vs. 74.79%, P = 0.01), but increased carcass yield in gilts (75.16 vs. 74.72%, P = 0.005) as compared to pigs fed the ractopamine diet without betaine; however, no effects of betaine were observed when supplemented to diets without ractopamine (data not shown).

Experiment 2

Average minimum temperature for the experimental barns was 26 ± 3°C and average maximum was 33 ± 3°C, average lowest temperature for the experimental barns was 21 ± 1°C and average highest temperature were 30 ± 4°C (Figure 2). Analyzed betaine concentrations were 0.005, 0.071, 0.134, and 0.200% for treatments formulated to contain 0.0, 0.0625, 0.125, and 0.1875% betaine, respectively. The diet without betaine and supplemented with ractopamine contained 0.010% betaine (Table 1).

Dietary treatments did not affect pig mortality (P = 0.701). The results of Exp. 2 did not demonstrate any effects (P > 0.05) of betaine on growth performance (Table 4). Supplementation of ractopamine increased market BW, ADG, and G:F by 3.09 kg, 102.4 g/d, and 38.9 g/kg, respectively ($P \leq 0.001$). Dietary treatments did not affect days to market, and barrows reached market earlier than gilts as observed in Expt. 1. Betaine tended to reduce carcass yield in a quadratic manner (P = 0.076) (Table 5). Moreover, ractopamine increased HCW, carcass yield, loin depth, and lean percentage by 3.38 kg, 0.90%, 2.70 mm, 0.62%, respectively, and reduced back fat by 1.57 mm (P < 0.001).

Discussion

The economic losses due to high ambient temperatures in finishing pigs are mainly due to slow growth, increased mortality, and decreased carcass quality (increased fat and reduced lean). Independently, betaine (Cadogan et al., 1993; Lawrence et al., 2002; Feng et al., 2006) and ractopamine (Watkins et al., 1990; Stoller et al., 2003; Lowe et al., 2014) have shown to improve growth performance and carcass quality in finishing pigs. In our experiment, we created high environmental temperatures to cause heat stress in pigs to evaluate the potential mutual benefits of supplemental betaine and ractopamine.

The upper critical temperature in pigs decreases with increasing pig BW and was estimated to be approximately 22°C in pigs weighing 90 kg (Renaudeau et al., 2011). Therefore, temperatures imposed in the current experiments were above the thermo-neutral zone and were expected to decrease growth performance and increase heat production, which are commonly associated with heat stress (Brown-Brandl et al., 2004; Renaudeau et al., 2011).

Betaine reduces maintenance energy requirements attributable to osmotic regulation, and thus increases energy availability for growth (Schrama et al., 2003). Supplementation of betaine to finishing pigs increased gain:feed, increased carcass yield, and decreased back fat (Sales, 2011). Greater benefits were observed when energy intake was restricted (Schrama et al., 2003; Dunshea et al., 2009). During heat stress, pigs reduce feed intake and experience hyperthermia, which are potential conditions for betaine to exert positive effects on growth performance. Ractopamine has consistently demonstrated to increase growth and lean deposition, while reducing feed intake when fed at 20 mg/kg (Apple et al., 2007). Ractopamine increased protein synthesis (Bergen et al., 1989) and allows for increased protein accretion (Webster et al., 2007). Reeds et al. (1985) estimated that 15 to 22% of the total energy expenditure of growing animals is due to muscle protein turnover. Indeed, the energy requirements for maintenance and heat production are positively correlated to body protein mass and are greater in lean pigs (de Lange et al., 2001; Brown-Brandl et al., 2004). In contrast, Yen et al. (1991) reported that supplementation with ractopamine did not impact fasting heat production that may have been related to decreased visceral organ weights (and associated reduction in heat production), offsetting increased heat production as a result of greater lean mass.

In Exp. 1, supplementation of betaine decreased growth performance and HCW, regardless of whether ractopamine was fed, but it did not affect lean or fat deposition. Dunshea et al. (2009) evaluated supplementation of betaine (0 or 0.15%) and ractopamine (0 or 10 ppm) in diets of gilts and boars during restricted-feeding (approximately 80 to 85% of ad libitum) and reported improved growth rate and feed efficiency in pigs fed betaine, which was more

pronounced when diets did not contain ractopamine. The authors further reported greater lean deposition and total lean mass in gilts fed betaine and these effects were additive with the supplementation of ractopamine. Under the heat-stress conditions applied in the current study, supplementation of betaine did not improve any aspects of growth performance or carcass characteristics in late finishing pigs regardless of whether ractopamine was fed or not. The reduction in growth rate due to betaine supplementation appeared to be a direct result of reduced feed intake. The effect of betaine on ADFI is variable (Sales, 2011) and may depend on the dietary lysine:calorie ratio (Matthews et al., 1998). Haydon et al. (1995) reported that 0.1% betaine fed to gilts (103 kg of BW) reduced ADFI when diets contained 2.59, and 2.80 g total Lys/Mcal DE but not when diets contained 1.88 and 2.09 g total Lys/Mcal DE. Similarly, Matthews et al. (1998) reported that pigs (111 kg BW) fed diets containing 0.125% betaine reduced ADFI when diets contained 2.2 g SID Lys/Mcal ME but not when they contained 1.9 g SID Lys/Mcal ME. Diets in the present study contained 2.21 and 2.93 g SID Lys/Mcal ME for treatments without and with ractopamine.

In Exp. 2, it was hypothesized that the level of betaine of 0.2% may have been too high to improve performance in pigs; thus, a dose titration was conducted to determine the optimal inclusion level of betaine. Inclusion of betaine in increments of 0.0625 up to 0.1875% did not impact any aspects of growth performance or carcass characteristics. Diets for this experiment were the same as for Exp. 1, with the same SID Lys/Mcal of ME ratio. In contrast to Exp. 1, no reduction in feed intake was observed, which may be related to the lower inclusion level of betaine. In a meta-analysis, Sales et al. (2011) reported that supplementation of betaine to finishing pigs decreased feed required per unit of weight gain (mean effect size of -0.361; 12

experiments), increased carcass yield (mean effect size of 0.358; 7 experiments), and decreased back fat thickness (mean effect size of -0.286; 10 experiments). However, under the heat stress conditions applied in the current studies, supplementation of betaine did not improve any aspects of growth performance or carcass characteristics in late finishing pigs when fed at various levels up to 0.20%.

Several studies have been conducted to determine the possible mode of action of betaine, including its role as a methyl donor, sparing methionine and choline, its impact on fat and energy metabolism, and the functional role as an osmolyte (Eklund et al., 2005; Ratriyanto et al., 2009). Thus, an excess or deficit of other nutrients or energy can impact the response of pigs to supplemental betaine. Consequently, the effect of dietary betaine on pig performance has been inconsistent (Ratriyanto et al., 2009; Sales, 2011). In the present study, diets were formulated to meet or exceed all nutrient requirements (NRC, 2012), ensuring that choline and methionine (methyl-donors) were provided in sufficient concentrations. Diets were based on corn, corn DDGS, and SBM, and these ingredients provided 1.3 g/kg of choline in the diet as compared to a requirement estimate for late finishing pigs of 0.3 g/kg (NRC, 2012). In addition, diets contained 0.30 and 0.31% of SID methionine for diets without and with ractopamine, which exceeds the requirements of 0.19% and 0.20% SID methionine (29% relative to SID lysine) for diets without and with ractopamine, respectively,as predicted by NRC (2012) for pigs weighing from 90 to 120 kg.

The effect of ractopamine on pig performance has been studied extensively (Bergen et al., 1989; Stoller et al., 2003; Lowe et al., 2014) and has consistently demonstrated to increase growth and lean tissue deposition (Apple et al., 2007). Pigs fed ractopamine have greater

dietary requirements for amino acid (and other nutrients) to accommodate for increased protein accretion (Schinckel et al., 2003). Supplementation of ractopamine increases the requirement for SID Lys by 26% (NRC, 2012). In the present study, diets with ractopamine contained 25% more SID Lys, 3% more SID Met, 7% more available P, and 3% more Ca. The increase in amino acids was accomplished by the addition of synthetic amino acids rather than increasing dietary SBM. In our experiments, we demonstrated that ractopamine improved growth performance and carcass characteristics under heat stress conditions and these responses were similar in magnitude as those reported previously (Apple et al., 2007).

Collectively, data indicate that under commercial conditions, betaine did not improve performance or carcass characteristics in pigs housed under high ambient temperatures, regardless of ractopamine inclusion. Ractopamine substantially improved pig growth and carcass characteristics under conditions of high ambient temperatures.

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Table 1. Composition of experimental diets for Exp. 1 and Exp. 2, as-fed basis[1]

	Exp. 1[2]			Exp. 2			
	Ractopamine HCl, ppm			Betaine, %[3]		Ractopamine HCl, ppm	
Ingredient, %	0	5.0	8.8	0	0.1875	5.0	8.8
Corn, 8.5% CP	62.62	61.98	61.96	62.62	62.29	61.78	61.76
Corn distillers dried grains with solubles	18	18	18	18	18	18	18
Soybean meal, 47.5% CP	15	15	15	15	15	15	15
Choice white grease	2.55	2.55	2.55	2.55	2.70	2.55	2.55
Limestone	1.00	1.02	1.02	1.00	1.00	1.02	1.02
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Monocalcium phospate, 21% P	0.18	0.23	0.23	0.18	0.18	0.23	0.23
Vitamin and mineral premix[4]	0.080	0.085	0.085	0.080	0.080	0.085	0.085
L-Lysine-HCl	0.165	0.485	0.485	0.165	0.165	0.485	0.485
L-Threonine	-	0.16	0.16	-	-	0.16	0.16
L-Tryptophan	-	0.05	0.05	-	-	0.05	0.05
DL-Methionine	-	0.015	0.015	-	-	0.015	0.015
Ractopamine[5]	-	0.025	0.045	-	-	0.025	0.045
Betaine[6]	-	-	-	-	0.1875	-	-
Red iron oxide[7]	-	-	-	-	-	0.2	0.2
Calculated Composition							
ME, Mcal/Kg	3.44	3.45	3.45	3.44	3.44	3.45	3.45
Total fat, %	7.22	7.22	7.22	7.22	7.36	7.20	7.20
CP, %	17.36	17.77	17.77	17.36	17.33	17.77	17.77
Lysine, %	0.88	1.13	1.13	0.88	0.88	1.13	1.13
SID Lys/ME, g/Mcal	2.21	2.93	2.93	2.21	2.21	2.93	2.93
SID Lys, %	0.76	1.01	1.01	0.76	0.76	1.01	1.01
SID SAA:Lys	0.75	0.58	0.58	0.75	0.75	0.58	0.58
SID Methionine, %	0.30	0.31	0.31	0.30	0.30	0.31	0.31
Ca, %	0.58	0.60	0.60	0.58	0.58	0.60	0.60
Available P, %	0.26	0.28	0.28	0.26	0.26	0.28	0.28
Analyzed composition, % as fed basis[8]							
CP	-	-	-	16.78	17.08	17.83	16.78
Crude fat	-	-	-	6.07	6.41	6.23	6.36
Crude fiber	-	-	-	12.15	12.09	11.88	11.98
Ca	-	-	-	0.52	0.49	0.47	0.48
P	-	-	-	0.42	0.4	0.43	0.45
Betaine (control/supplemented)	0/0.192	0/0.196	0/0.176	0.005	0.200	0.010	0.010

[1]Diets were formulated to meet or exceed NRC (2012) nutrient recommendations.
[2]Betaine was added at the expense of corn at 0.2% to diets containing 0, 5.0, and 8.8 ppm of ractopamine HCl.
[3]Diets with 0 and 0.1875% betaine were blended using a Howema system (Big Dutchman, Vechta, Germany) to create the intermediate diets containing 0.0625 and 0.1250% of betaine.
[4]Supplied per kg of complete diet (at the 0.08% inclusion rate): Zn, 96 mg as zinc oxide; Fe, 60 mg as ferrous sulfate; Mn, 24 mg as manganous oxide; Cu, 9.6 mg as copper sulfate; I, 0.56 mg as potassium iodide; Se, 0.24 mg as selenium selenite; vitamin A, 5,291 IU; vitamin D_3, 882 IU; vitamin E, 26.5 IU; vitamin K, 2.65 mg; vitamin B_{12}, 21.2 µg; riboflavin, 4.23 mg; d-pantothenate, 14.11 mg; niacin, 21.16 mg; and phytase, 529 phytase units (Phyzyme, Danisco A/S, Copenhagen, Denmark).
[5]Ractopamine hydrochloride (Paylean 9, Elanco Animal Health, Greenfield, IN) added at 5 ppm for 21 d followed by 8.8 ppm until pigs reached market weight.
[6]Natural betaine (Betafin, Danisco A/S, Copenhagen, Denmark).
[7]Used to color code experimental diets.
[8]Analyzed by the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO. Betaine was analyzed by Eurofins (Eurofins Scientific Inc., Des Moines, IA). In Exp 2 analyzed concentrations were 0.0706, and 0.1335% of betaine for dietary treatments containing 0.0625 and 0.1250 % of betaine.

Table 2. Effects of supplementation of betaine and ractopamine on growth performance of pigs (Exp. 1)[1]

	Betaine, %					Sex			P-values[3]		
	0		0.2								
	Ractopamine, ppm[2]										
Item	0	5/8.8	0	5/8.8	SEM	Barrows	Gilts	SEM	Betaine	Ractopamine	Sex
Body Weight, kg											
d 0	91.9	92.0	91.9	92.0	0.822	92.7	91.2	1.150	0.909	0.426	0.362
d 27[4,5]	113.77	117.42	112.62	115.89	0.380	116.06	113.79	0.301	0.001	<0.001	<0.001
Market[4,6]	122.36	126.26	121.38	124.80	0.469	122.99	124.41	0.394	0.008	<0.001	0.023
ADG, kg											
d 0 to d 27[7]	0.835	0.975	0.797	0.915	0.014	0.940	0.821	0.011	0.001	<0.001	<0.001
d 0 to market	0.778	0.865	0.759	0.800	0.021	0.846	0.755	0.015	0.048	0.003	<0.001
ADFI (as fed basis), kg											
d 0 to d 27[4]	2.755	2.718	2.660	2.588	0.030	2.856	2.504	0.021	0.001	0.074	<0.001
d 0 to market[4]	2.951	2.885	2.840	2.759	0.034	3.030	2.688	0.024	0.001	0.033	<0.001
G:F											
d 0 to d 27[4,7]	0.303	0.359	0.300	0.354	0.003	0.329	0.328	0.002	0.198	<0.001	0.735
d 0 to market[4]	0.263	0.300	0.267	0.290	0.006	0.279	0.281	0.004	0.603	<0.001	0.823
Days to Market[8]	39.89	39.80	39.97	39.89	0.108	37.14	42.63	0.076	0.443	0.463	<0.001
Mortality, %	1.09	0.85	0.56	1.58	0.551	1.11	0.94	0.389	0.841	0.494	0.773

[1]Values represent least squares means of 17 pens with 20 to 23 pigs per pen for dietary treatment effects, and least squares means of 34 pens with 20 to 23 pigs per pen for sex effect.

[2]Ractopamine was fed at a concentration of 5 ppm for 21 d followed by 8.8 ppm until pigs reached market weight.

[3]The statistical analysis tested for main effects of betaine, ractopamine, sex and their interactions as fixed effects. Weight block nested within sex was used as random effect.

[4]Initial body weight was used as a covariate for the statistical analysis ($P \leq 0.04$)

[5]All pigs were weighted at d 27 of being on test, which was immediately prior to marketing at the desired market BW of approximately 125 kg.

[6]Pigs within pen were shipped to the packing plant when they reached market weight in 3 to 4 groups over the course of 3 weeks.

[7]Betaine and Sex interaction ($P = 0.02$). Mean G:F were 0.335 and 0.324 for control and betaine, respectively for barrows and 0.326 and 0.330 for gilts.

[8]Days to market consists of the weighted average per pen of the number of days from initiation of the study until reaching market weight.

Table 3. Effects of supplementation of betaine and ractopamine on carcass characteristics of pigs (Exp. 1)[1]

Item	Betaine, %				SEM	Sex		SEM	P-values[3]		
	0		0.2								
	Ractopamine, ppm[2]										
	0	5/8.8	0	5/8.8		Barrows	Gilts		Betaine	Ractopamine	Sex
Hot carcass weight, kg	90.31	94.66	89.75	93.57	0.747	91.94	92.20	0.921	0.056	<0.001	0.842
Carcass yield, %	73.70	74.75	73.81	74.81	0.136	74.13	74.41	0.096	0.533	<0.001	0.052
Back fat depth, mm[4, 5]	22.24	20.25	22.66	20.16	0.266	23.18	19.48	0.190	0.524	<0.001	<0.001
Loin depth, mm[4, 5]	59.75	63.66	60.22	63.45	0.394	61.09	62.45	0.263	0.725	<0.001	0.002
Lean percentage, %[4, 5, 6]	51.72	53.18	51.66	53.18	0.119	51.64	53.22	0.081	0.801	<0.001	<0.001

[1]Values resent least squares means of 17 pens with 20 to 23 pigs per pen for dietary treatment effects, and least squares means of 34 pens with 20 to 23 pigs per pen for sex effect.

[2]Ractopamine was fed 5 ppm for 21 d followed by 8.8 ppm until pigs reached market.

[3]The statistical analysis tested for main effects of betaine, ractopamine, sex and their interaction as fixed effects, block nested within sex was used as ramdom effect.

[4]The probe was inserted into the carcass approximately between the 3rd and 4th last rib at a distance of approximately 7 cm from the midline.

[5]Hot carcass weight was used as a covariate for the statistical analysis ($P < 0.01$).

[6]Lean percentage was determined by the equation used at Triumph Foods packing plant (St. Joseph, MO). Lean percentage = 100 x (2.827 + (1.0340 x HCW, kg) - (0.7272 x backfat, mm) + (0.3868 x loin depth, mm))/(2.2046 x HCW, kg).

Table 4. Effects of supplementation of betaine and ractopamine on growth performance of pigs (Exp.2)[1]

Item	Betaine, %				Ractopamine[2]	SEM	Sex		SEM	P-value[3]	
	0	0.0625	0.125	0.1875			Barrows	Gilts		Diet	Sex
Body Weight, kg											
d 0	95.6	95.6	95.5	95.6	95.6	0.776	94.3	96.8	1.059	0.997	0.109
d 21[4]	109.28[a]	108.76[a]	108.87[a]	108.9[a]	111.05[b]	0.887	107.26	111.48	1.130	0.001	0.017
market[5]	118.58[a]	118.78[a]	118.30[a]	118.43[a]	121.61[b]	0.687	117.82	120.46	0.601	0.001	0.006
ADG, kg											
d 0 to d 21	0.703[a]	0.677[a]	0.687[a]	0.684[a]	0.793[b]	0.018	0.719	0.699	0.011	<0.001	0.227
d 0 to market	0.730[a]	0.721[a]	0.717[a]	0.729[a]	0.827[b]	0.018	0.719	0.769	0.012	<0.001	0.008
ADFI (as fed basis), kg											
d 0 to d 21	2.419	2.379	2.452	2.388	2.465	0.041	2.491	2.350	0.038	0.333	0.016
d 0 to market	2.445	2.445	2.481	2.451	2.487	0.040	2.521	2.402	0.035	0.834	0.029
G:F											
d 0 to d 21	0.290[a]	0.285[a]	0.279[a]	0.286[a]	0.323[b]	0.006	0.289	0.297	0.004	<0.001	0.116
d 0 to market	0.298[a]	0.295[a]	0.289[a]	0.298[a]	0.334[b]	0.006	0.286	0.320	0.005	<0.001	<0.001
Days to market, d[6]	31.34	31.39	31.34	31.32	31.18	0.500	32.24	30.39	0.607	0.989	0.045
Mortality, %	0.65	0.23	0.88	0.66	0.89	0.367	1.14	0.18	0.24	0.701	0.012

[1]Values represent least squares means of 20 pens with 20 to 24 pigs per pen for dietary treatment effects, and least squares means of 50 pens with 20 to 24 pigs per pen for sex effect.

[2]Ractopamine was fed 5 ppm for 21 d followed by 8.8 ppm until reach market.

[3]The statistical analysis tested for main effects of dietary treatment, sex, and their interaction as fixed effects, weight block nested within sex was used as random effect.

[4]All pigs were weighted at d 21 of being on test, which was immediately prior to marketing at the desired market BW of approximately 120 kg .

[5]Pigs within the pen were marketed in 3 or 4 groups over the course of 3 weeks until there were no pigs remaining in the pen.

[6]Days to market consists of the weighted average per pen of the number of days from initiation of the study until reaching market weight.

[ab]Within a row, means without a common superscript differ ($P \leq 0.05$) using the Tukey's method for multiple comparisons.

Table 5. Effects of supplementation of betaine and ractopamine on carcass characteristics of pigs (Exp.2)[1]

Item	Betaine, %				Ractopamine[2]	SEM	Sex		SEM	P-value[3]	
	0	0.0625	0.125	0.1875			Barrows	Gilts		Diet	Sex
Hot carcass weight, kg	87.83[a]	87.34[a]	87.28[a]	87.51[a]	90.87[b]	0.547	87.62	88.71	0.586	<0.001	0.206
Carcass yield, %[4]	74.09[ab]	73.54[a]	73.78[a]	73.89[a]	74.73[b]	0.208	74.37	73.64	0.182	<0.001	0.012
Back fat, mm[5,6]	20.26[a]	20.30[a]	20.31[a]	20.52[a]	18.78[b]	0.246	22.68	17.39	0.153	<0.001	<0.001
Loin depth, mm[5]	59.48[a]	58.62[a]	58.73[a]	59.11[a]	61.68[b]	0.484	58.71	60.34	0.355	<0.001	0.005
Lean percentage, %[6,7]	52.61[a]	52.45[a]	52.46[a]	52.45[a]	53.53[b]	0.150	51.63	53.77	0.128	<0.001	<0.001

[1]Values represent least squares means of 20 pens with 20 to 24 pigs per pen for dietary treatment effects, and least squares means of 50 pens with 20 to 24 pigs per pen for sex effect.
[2]Ractopamine was fed 5 ppm for 21 d followed by 8.8 ppm to market
[3]The statistical analysis tested for main effects of dietary treatment, sex, and their interaction as fixed effects, weight block nested within sex was used as random effect.
[4]Quadratic effect of betaine ($P = 0.076$).
[5]The probe was inserted into the carcass approximately between the 3rd and 4th last rib at a distance of approximately 7 cm from the midline.
[6]Hot carcass weight was used as a covariate for the statistical analysis ($P < 0.001$).
[7]Lean percentage was determined by the equation used at Triumph Foods packing plant (St. Joseph, MO). Lean percentage = (2.827 + (1.0340 * HCW, kg) - (0.7272 x back fat, mm) + (0.3868 x loin depth, mm)))/ 2.20462 *HCW, kg.
[ab]Within a row, means without a common superscript differ ($P \leq 0.05$) using the Tukey's method for multiple comparisons.

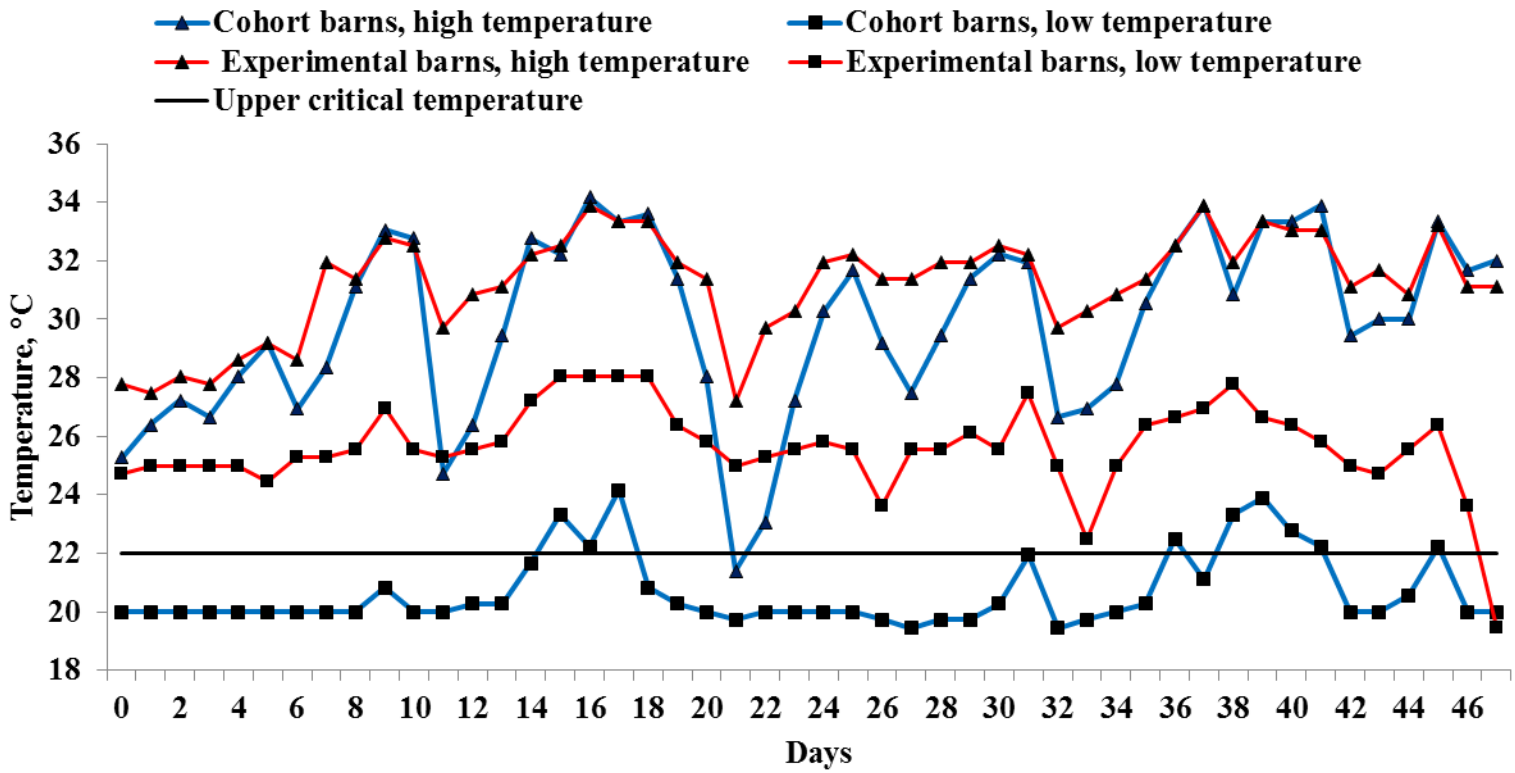

Figure 1. High and low temperatures Exp. 1. Average lowest temperature for the experimental barns was 26 ± 1°C and average highest temperature were 31 ± 2°C. Average lowest temperature for the cohort barns was 21 ± 1°C and average highest temperature were 30 ± 3°C. The upper critical temperature for a 90 kg BW pigs is estimated to be 22°C (Renaudeau et al., 2011).

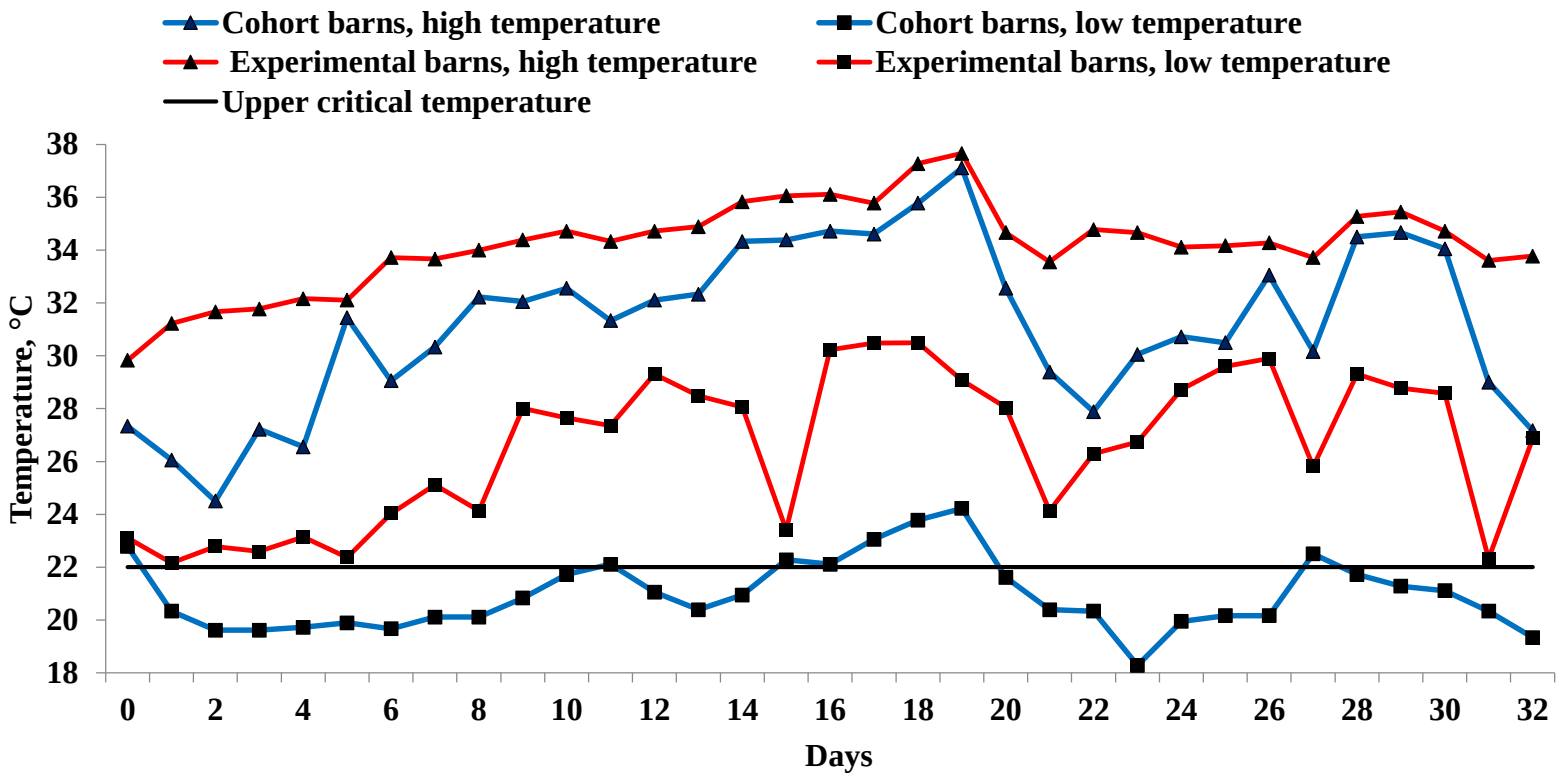

Figure 2. Average minimum temperature for the experimental barns were 26 ± 3°C and average maximum was 33 ± 3°C. Average minimum temperature for the cohort barns were 21 ± 1°C and average maximum was 30 ± 4°C. The upper critical temperature for a 90 kg BW pigs is estimated to be 22°C (Renaudeau et al., 2011).

CHAPTER IV:

Sow performance in response to natural betaine fed during lactation and post-weaning during summer and non-summer month

Abstract: Two studies were conducted to evaluate the effects of dietary betaine during lactation and weaning-to-35 d post-insemination on sow reproductive performance during summer (experiment 1) and non-summer months (experiment 2). Both studies were designed as a 2 x 2 factorial arrangement. Factors included dietary betaine (0 or 0.2%) and period of supplementation (lactation or post-weaning until 35 d post-insemination). In Exp.1 a total of 169, 153, and 327 sows representing parity 1, 2, and 3 to 6 (P3+) were used. In Exp. 2 a total of 250, 50, and 327 sows representing parity 1, 2, and 3 to 6 (P3+) were used. Lactation diets were corn-soybean meal based with 10% rice bran, 6.0% wheat middlings, and contained 1.9 g of choline/kg of diet, 3.31 g SID Lys/Mcal ME and a SID Met+Cys:Lys ratio of 0.56. Post-weaning diets were corn-soybean meal based with 30% wheat middlings, 15% rice bran, and contained 1.15 g of choline/kg of diet, 1.82 g SID Lys/Mcal ME and a SID Met+Cys:Lys ratio of 0.69. Data were analyzed using the MIXED procedure of SAS and GLM was used for dichotomous variables. In Exp. 1, supplementation of betaine during lactation increased sow BW losses (-11.95 vs. -14.63 kg; $P = 0.024$) and reduced ADFI (4.28 vs. 4.12 kg/d $P = 0.052$). No differences in litter gain or the number of pigs weaned were observed due to betaine supplementation ($P = 0.535$). Supplementation of betaine during post-weaning reduced the weaning to estrus interval (5.75 vs. 6.68 d, $P = 0.054$), regardless of parity group, and reduced farrowing rate in P3+ sows (75.90 vs. 89.00%), but not P1 and P2 sows (interaction, $P = 0.078$). Betaine supplementation during lactation in P4+ sows increased total pigs born (15.37 vs. 14.15; $P = 0.08$) but numerically reduced farrowing rate (85.5 vs. 92.2%) compared to sows that did not received betaine in any of the periods. Betaine supplementation in P1 sows in the post-weaning period increased total pigs born (13.81 vs. 12.32; $P = 0.08$) but numerically

reduced farrowing rate (75.0 vs. 79.5 %) as compared to sows that did not received betaine in any of the periods. Betaine did not affect litter size in P2 or P3 sows ($P \geq 0.550$). In Exp. 2, betaine did not affect sow or litter performance during the lactation period ($P \geq 0.155$). Supplementation of betaine during lactation reduced the weaning to estrus interval (6.64 vs. 7.50; $P = 0.077$) and farrowing rate in P3+ sows (71.3 vs. 85.9 %; interaction, $P = 0.054$). Supplementation of betaine during the post-weaning period reduced the total number of pigs born (13.00 vs. 13.64; $P = 0.04$) and pigs born alive (12.30 vs. 12.82; $P = 0.075$), regardless of parity group. The use of 0.2% betaine during the non-summer months did not appear to benefit sow performance and subsequent litter size. During the summer months, betaine supplementation in lactation and the post-weaning period increased subsequent litter size in P1 and P4+ sows, respectively. However, further research is needed to determine if the numeric reduction in farrowing rate is persistent and depends on level of betaine inclusion.

Key words: betaine, sow, lactation, post-weaning.

Introduction

Sow reproductive performance severely declines during the summer season. It is estimated that during summer months sow non-productive days increase by 5 to 19 days (St. Pierre et al., 2003). Non-reproductive days mainly occur due to a delay in the estrus, failure to return to estrus, or embryo losses after mating (Hennessy and Williamson, 1984). During summer months, farrowing rate has been reported to be 20% lower than sows bred during non-summer months (Hennessy and Williamson, 1984). In addition, total number of pigs born is

estimated to be reduced by 0.05 piglet for each °C above 20°C on the day of insemination (Bloemhof et al., 2013).

The negative effects of heat stress on sow reproduction also cause greater BW losses during the lactation period. Above 25°C, sows decrease feed intake by 0.5 kg/d per additional degree °C (Quiniou and Noblet, 1999) and increase tissue mobilization to sustain milk production (Spencer et al., 2003). Sows with poor feed intake during lactation continue the subsequent reproductive period with a negative energy balance (Black et al., 1993), which acts as a negative feedback to prevent the onset of a new reproductive cycle.

Betaine, or tri-methyl glycine, is a methyl donor and osmoregulator that naturally accumulates in organisms that are adapted to saline or drought conditions (Kidd et al., 1997; Ashraf and Foolad, 2007). The role of betaine as an osmolyte has important implications because it can maintain cell volume and integrity under challenging conditions. During heat stress, body adaptation causes ischemia and hypoxia in the gastrointestinal organs (Cronjé, 2005). These events disrupt ion homeostasis and cause cell damage, especially in enterocytes and hepatocytes (Hall et al., 1999). The osmolyte betaine has shown to prevent cell damage in heat stress rats (Wettstein and Haussinger, 1997). In addition, supplementation of betaine to heat-stressed broilers reduces mortality (Zulkifli et al., 2004) and improves growth performance (Farooqi et al., 2005; Akhavan-Salamat and Ghasemi, 2016). Dietary supplementation to heat-stressed rabbits reduces hyperthermia and improve growth (Hassan et al., 2011).

Ramis et. al (2010) fed betaine to sows during lactation and reported an improvement in litter gain. In addition, dietary betaine supplementation to lactating sows reduced the

weaning to estrus interval (Ramis et al., 2011; Greiner et al., 2014; Cabezon et al., 2016) and increased subsequent litter size (Campbell and Virtanen, 2001; Greiner et al., 2014). Van Wettere et al. (2012) reported that feeding betaine to gestating sows during summer months increased litter size, in particular, in mature sows.

We propose that the osmolyte betaine can alleviate the negative effects caused by high temperature in sows, by reducing the weaning to estrus interval, and increasing farrowing rate and subsequent litter size. The objective of the current studies was to evaluate the impact of dietary betaine fed during lactation and post-weaning as a heat abatement strategy in sows exposed to heat stress

Material and Methods

Two studies were conducted in a commercial research facility in Oklahoma (2,600 sows). Sows (Camborough, PIC, Hendersonville, TN) and piglets (TR-4 x Camborough product sows, Pig Improvement Company, Hendersonville, TN) used in the experiments were humanely treated following the practices outlined in the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). Protocols were under the supervision of licensed veterinarians.

The two studies had the same experimental design and used the same diet formulations with the difference being the season that they were conducted. The design was a 2 x 2 factorial arrangement. Factors included dietary betaine (Vistbet®, AB Vista, Malborough, UK) supplemented at 0 or 0.2% and 2 periods of supplementation (lactation or post-weaning until

35 d post-insemination). Dietary treatments were initiated immediately after farrowing and sows were fed lactation diets with either 0 or 0.2% betaine. After weaning, sows fed 0% betaine during lactation were either fed 0 or 0.2% betaine from weaning until 35 d post-breeding and, similarly, sows fed 0.2% betaine during lactation were fed either 0 or 0.2% betaine post-weaning until 35 d post-breeding. Diets were formulated to meet or exceed NRC (2012) nutrient recommendations and manufactured by a commercial feed mill (Hanor Company, Enid, Oklahoma). Lactation diets (Table 1) were formulated to contain 3.31 g SID Lys/Mcal ME, 0.56 SID Met+Cys:Lys ratio, and 1.9 g of choline/kg of diet. Diets for the post-weaning period were formulated to contain 1.82 g SID Lys/Mcal ME, 0.69 SID Met+Cys:Lys ratio, and 1.15 g of choline/kg of diet (Table 1). Diets were formulated to supply sufficient methyl donors to specifically evaluate osmolyte properties of betaine. For both diets, the inclusion of betaine was made at the expense of corn. Diets were color coded to visually confirm that proper diets were fed to the correct treatment groups. Feed samples were collected at the feed mill for every batch and every week at the farm to chemically verify the diets.

The proximate analysis (Table 2) of the diets was conducted by the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) using AOAC (2005) procedures. Diets were analyzed for CP (Method 990.03), crude fat (Method 920.39 (A)), crude fiber (Method 978.10), and ash (Method 942.05).

During the course of the experiments, ambient temperature and humidity of the farrowing room and breeding barn were recorded using data recorders (logtag, MicroDAQ Ltd., Contoocook, NH). Data recorders measured temperature and humidity every 10 minutes.

Experiment 1

A total of 649 sows entered the farrowing room in groups of 20 to 26 sows per group. Within each group, sows were randomly assigned within parity groups to 1 of 4 treatments. The experimental design consisted of a generalized randomized complete block design and was balanced by parity. A total of 169, 153, and 327 sows representing parity 1, 2, and 3 to 6 (P3+) were used in the study. The first group entered the farrowing room in June and the last group (30^{th}) was weaned in September. The first group was weaned in July and the last group was relocated to the gestation barn in November (d 35 post-insemination).

Experiment 2

A total of 627 sows entered the farrowing room in groups of 22 to 24 sows per group. Within each group, sows were randomly assigned to 1 of 4 treatments considering parity groups. The experimental design consisted of the generalized randomized complete block design and was balanced by parity. A total of 250, 50, and 327 sows representing parity 1, 2, and 3 to 6 (P3+) were used in the study. The first group entered the farrowing room in February and the last group (28^{th}) was weaned in June. The first group was weaned in March and the last group was relocated to the gestation barn in July (d 35 post-insemination).

Lactation period

Sows were weighed individually when they entered the farrowing room. In Exp.1 sows entered the farrowing room on d 109 ± 1 and in Exp. 2 on d 112 ± 2 of gestation. Between placement and farrowing, sows were fed 1.82 kg/d of 0% betaine lactation diet. After farrowing feed addition and feed refusal of the 2 lactation treatments were recorded from the day the

sows farrowed until weaning. Sows were fed to satiety and feed was offered at 0800 and 1400 h to appetite, ensuring sows had some feed in front of them at all times. Number and weight of pigs at birth (alive, still born, and mummies) were recorded and placenta and weight of fluids were calculated from the equations reported by (Walker and Young, 1993). Litter birth weight, estimated placenta weight and estimated weight of fluids were subtracted from the weight of the sow at placement to estimate sow weight at farrowing. Sows were weighed for a second time at d 21 of lactation. The difference between sow weight at d 21 and farrowing weight represents the body weight loss (or gain) during lactation. Sow G:F was calculated as total gain during lactation (sow body weight gain or loss plus total litter gain) divided by total feed intake during lactation

Cross-fostering was done 18 to 24 h after farrowing to allow for colostrum intake by piglets from their own mothers. Litter size was standardized to 12 pigs. Initial litter weight was recorded to estimate litter gain (Exp. 1, n = 649; Exp. 2, subset n = 352). Handling, processing, and vaccination of piglets were performed according to the recommendations of licensed veterinarians and were identical for all litters. Litter weight gain was calculated as the difference between the weight of the litter at d 21 and the weight of the litter after cross-fostering. Date and weight of dead piglets were recorded. The piglets that did not reach 3.62 kg of BW on d 21 were considered no-value pig. Pigs did not receive creep feed or milk replacer during the experiment. Piglets were weaned at the first opportunity after d 21 following the production schedule (22 ± 1 d in Exp. 1; 23 ± 1 d in Exp. 2).

Rectal temperature and respiration rate were measured on d 18 of lactation in a subset of sows (n = 44 in Exp. 1, n = 56 in Exp. 2). Measurements were taken between 1600 h and

1800 h. Rectal temperature was measured by using a digital thermometer (M750 Series, GLA Agriculture Electronics, San Luis Obispo, CA). Respiration rate was measured as the number of flank movements per 30 s.

Post-weaning to 35 d post-insemination

The breeding barn at the farm facilities is equipped with drop feeders (capacity of 3.62 kg) and has two independent feeding systems. One-half of the barn received the 0% betaine and the other half the 0.2% betaine post-weaning diets. After weaning, sows were distributed in the barn according to their assigned treatment and maintained until d 35 post insemination. Sows had ad libitum access to feed before signs of estrus were detected. At that point, sows were artificially inseminated and feed drops were adjusted to offer 1.8 to 2.7 kg of feed depending on sow body condition. Feed was delivered in two meals. In Exp. 1 feed was delivered at 0200 h and 0800 h, and in Exp. 2 feed was delivered at 0900 h and 1200 h. The schedule followed the recommendations to avoid high temperatures during the summer months.

Estrus detection and artificial insemination were performed following the standard operating procedures of the farm. Sows that did not return to estrus within 14 d discontinued the dietary treatments and were not used in the final analysis for subsequent litter size. After d 35 post-insemination, sows were moved to the gestation barn and were fed the standard gestation diet (control without betaine). Data collection during this period included: days to estrus, the number of sows bred, the number of sows bred within 14 d after weaning, sows that farrowed, and sows that were culled. Once sows reached approximately d 110 of gestation,

they were moved to the farrowing room and subsequent litter size at birth was recorded (born alive, still born, and mummies).

Statistical Analysis

Sow and litter performance data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the effect of betaine in lactation, parity group, and their interaction as fixed effects. The groups of sows (Exp. 1 groups = 30; Exp. 2. Groups = 28) that entered the farrowing room together were considered as the random effect. Rectal temperature and respiration rate were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the effect of betaine in lactation, parity group, and their interaction as fixed effects. The sow was considered as the random effect.

Subsequent reproductive performance data were analyzed using the MIXED procedure of SAS for days to estrus, total born, born alive, still born and mummies. The GLM procedure of SAS was used for dichotomous variables. The model included the effect of betaine during lactation, the effect of betaine post-weaning, parity group, and their interaction as fixed effects. Groups of sows were considered as random effect for days to estrus, total born, born alive, still born and mummies. Least squares means were reported, and differences were considered statistically significant at $P \leq 0.05$ and were considered tendencies when $0.05 < P \leq 0.10$. The least significant difference method was used for single degree of contrast multiple comparisons.

Results

Experiment 1

The average temperature of the farrowing room during the lactation period was 25.2 ± 2ºC and relative humidity was 72.5± 4%. The average temperature of the breeding barn (during the post-weaning until 35 d post-insemination period) was 24.1± 2ºC and relative humidity was 73.5± 6%.

As expected, P3+ sows had greater BW at placement, after farrowing, and at d 21 of lactation ($P < 0.001$) (Table 3). BW losses were greater for P1 and P2 sows than P3+ ($P < 0.001$). Average daily feed intake and sow G:F were greater for P3+ ($P < 0.001$). Litter gain was greater for piglets from P3+ sows ($P < 0.001$), but no differences in the number of pigs weaned or mortality were observed due to parity group ($P \geq 0.358$). Supplementation of betaine during the lactation period increased BW losses ($P = 0.024$) and this was related to a lower ADFI ($P = 0.052$). No differences in litter gain ($P = 0.350$) or the number of pigs weaned were observed ($P = 0.535$) due to betaine supplementation. However, supplementation of betaine tended to reduce the percentage of no-value pig ($P = 0.071$).

The average daily temperature of the farrowing room was 26.56 ±2°C when rectal temperature and respiration rate were measured in the subset of sows. Betaine supplementation in lactation did not affect rectal temperature or respiration rate ($P \geq 0.211$) (Table 4).

Following the lactation period, young sows (P1 and P2) tended to have greater weaning to estrus interval ($P = 0.068$) than mature sows (P3+). In addition, more P1 and P2 sows tended to be sold as cull sows ($P = 0.098$) (Table 5). A greater number of P3+ sows were bred within

14 d after weaning (bred within 14 d: weaned) and overall (bred:weaned) ($P \leq 0.037$). Mature sows (P3+) had a greater number of total pigs born and pigs born alive than P1 and P2 sows ($P \leq 0.026$). In addition, P3+ sows had greater number of still born pigs ($P = 0.001$).

Supplementation of betaine post-weaning tended to reduce ($P = 0.056$) the number of days to estrus. Supplementation of betaine during lactation to P1 and P2 sows reduced the number of sows bred (bred:weaned), but not in P3+ sows (interaction, $P = 0.004$). Supplementation of betaine during lactation or post-weaning did not affect the number of sows bred within 14 days after weaning (bred within 14 d:weaned) ($P \geq 0.127$). Supplementation of betaine during lactation to P1 and P2 sows reduced the number of sows that returned to estrus after being bred (returns:bred with 14 d), but betaine during lactation to P3+ sows increased the number of sow that returned to estrus after being bred (interaction, $P = 0.058$).

Supplementation of betaine during the post-weaning period to P3+ sows reduced the number of sows that farrowed ($P = 0.078$) but did not affect P1 and P2 sows. A tendency for a three-factor interaction was observed for the total number of pigs born ($P = 0.087$) and pigs born alive ($P = 0.058$). Mature sows (P3+) when they received betaine during the post-weaning period had a reduced number of total pigs born and pigs born alive while P1 and P2 sows had a reduced total number of pigs born and pigs born alive when receiving betaine in both periods or when they did not receive betaine in any of the periods.

An exploratory analysis was conducted to further separate the parity groups and determine the impact of betaine within these parity groups (Table 6). For P1 sows, supplementation of betaine did not affect farrowing rate ($P \geq 0.195$). Parity 1 sows reported an interactive effect of betaine supplementation during the lactation and post-weaning periods on

the number of total pigs born (*P = 0.025*) and pigs born alive (*P* = 0.007). Supplementation of betaine to P1 during the post-weaning period increased total number of pigs born and pigs born alive. However, supplementation of betaine during the lactation and subsequently fed in the post-weaning period reduced total number of pigs born and pigs born alive. For parity 2 and 3 supplementation of betaine in the post-weaning period reduced farrowing rate (*P* = 0.052) but did not affect total number of pigs born or pigs born alive. For parity 4 or greater (4, 5, and 6) supplementation of betaine post-weaning reduced farrowing rate (*P* = 0.004), total number of pigs born (*P* = 0.014) and pigs born alive (*P* = 0.084). Supplementation of betaine during lactation increased total number of pigs born (*P* = 0.026) and did not affect farrowing rate (*P* = 0.534).

Experiment 2

The average temperature of the farrowing room during the lactation period was 23.2 ± 2ºC and relative humidity was 69.8± 8%. The average temperature of the breeding barn (during the post-weaning until 35 d post-insemination period) was 23.8± 2ºC and humidity was 68.7± 12%.

Mature sows (P3+) had greater (*P* < 0.001) BW at placement, after farrowing, and at d 21 of lactation (Table 7). During the lactation period, P3+ sows increased BW, while P1 and P2 had BW losses (*P* < 0.001). Average daily feed intake and sow G:F were greater for P3+ sows (*P* < 0.001). Litter gain was greater for P3+ sows (*P* < 0.001), but there were no differences in the number of pigs weaned or mortality due to parity group (*P* ≥ 0.816). Supplementation of betaine during the lactation period did not affect sow and litter

performance ($P \geq 0.155$).

The average daily temperature of the farrowing room was and 23.61°C±1 when rectal temperature and respiration rate were measured in the subset of sows. Betaine supplementation in lactation did not affect rectal temperature and respiration rate ($P \geq 0.789$).

A greater number of P3+ sows returned to estrus ($P = 0.08$) after being bred (returns:bred with 14 d) compared to P1 and P2 sows (Table 8). A greater number of P1 and P2 sows were sold as cull sows compared to P3+ sows ($P = 0.069$). Mature sows (P3+) had a greater number of total pigs born, pigs born alive, still born pigs ($P \leq 0.001$), and mummies ($P = 0.073$) compared to P1 and P2 sows.

A tendency for a three-factor interaction was observed for days to estrus ($P = 0.09$). Supplementation of betaine during either lactation or post-weaning or in combination (lactation and post-weaning) reduced the number of days to estrus in P3+ sows. Supplementation of betaine in both periods (lactation and post-weaning) reduced days to estrus in P1 and P2 sows. As an overall effect, betaine supplementation during lactation tended to reduce days to estrus ($P = 0.077$).

Supplementation of betaine during the post-weaning period to P1 and P2 sows reduced the number of sows bred (bred:weaned) (interaction, $P = 0.078$) but did not affect P3+ sows. Supplementation of betaine during lactation to P3+ sows increased the number of sows that returned to estrus after first insemination (returns:bred within 14 d and returns:bred; interaction, $P = 0.040$ and $P = 0.036$, respectively) but did not affect P1 and P2 sows. Supplementation of betaine during lactation to P3+ reduced the number of sows that farrowed (interaction, $P = 0.008$) but did not affect P1 and P2 sows. The reduction in number of P3+

sows that farrowed when fed betaine in lactation was driven by an increase in the number of sows sold as cull sows (interaction, $P = 0.1$). In this group of 23 sows, 20 sows were sold as cull sows due to reproductive reasons. Supplementation of betaine in the post-weaning period reduced total number of pigs born ($P = 0.004$) and pigs born alive ($P = 0.075$), regardless of parity group.

Discussion

Dietary supplementation of betaine has shown to improve the subsequent reproductive performance of sows when fed during lactation and gestation in summer months. In the present studies, we evaluated betaine supplementation during the lactation and post-weaning until d 35 post-insemination period to determine the optimum time frame for betaine supplementation. In addition, we evaluated which parity of sows were more responsive to betaine supplementation because previous data suggested that mature sows had a greater response than young sows. Two studies were conducted, one during the summer months and one during non-summer months to determine if the effect of betaine was specific during the summer season or could also be detected during the non-summer season.

The diets in the present studies were formulated to provide enough choline and methionine to supply sufficient quantities of methyl donors in order to determine the impact of the osmolyte properties of betaine. The choline requirement for lactating sows is 5.25 g/d and for gestating sows is 2.3 g/d (NRC, 2012). During the lactation period, sows were provided with 8.74 g/d of choline and during the post-weaning period with 2.5 g/d of choline.

Methionine requirements for lactating and gestating sows are 0.22 and 0.12% of standardized ileal digestible (SID) methionine, respectively (NRC, 2012). Diets in the present studies were formulated to provide 0.28 and 0.18% of SID methionine, respectively. The concentration of betaine evaluated in the present experiments based on Campbell and Virtanen (2001), who reported that betaine supplementation at 0.2% increased the number of pigs in the subsequent cycle by 1 pig, whereas betaine supplemented at 0.4% reduced sow feed intake..

Experiment 1 was conducted during summer months; the average temperature in the farrowing room was 2 degrees Celsius greater than the average temperature recorded in the non-summer experiment and 1 degree Celsius greater in the breeding barn. Thus, temperatures in the summer experiment were only slightly higher than the non-summer experiment. Nonetheless, sows in the summer experiment had a respiration rate that was 50% greater than sows in the non-summer experiment, but rectal temperatures were not markedly different.

In the summer experiment, sows were 13 kg heavier when entering the farrowing rooms than the sows in the non-summer experiment. Nonetheless, BW at d 21 were similar in both experiments. Therefore, BW losses were greater in the summer experiment, which was associated with a reduction in feed intake of 14%. Surprisingly, in the summer experiment, litter gain was 13% greater and sows weaned 0.2 more pigs compared to the non-summer experiment.

Supplementation of betaine during lactation in the summer experiment reduced ADFI, but the reduction in ADFI was not observed in the non-summer study. Reduction in feed intake in lactating sows has been observed when anhydrous betaine was added at 0.2% (Ramis et al., 2011) and at 0.4% (Campbell and Virtanen, 2001). However, Cabezon et al. (2016) reported

an increase in ADFI when 0.3% of betaine hydrochloride (70.7% betaine) was added to diets of lactating sows. The effect of betaine on ADFI of swine is variable; betaine can increase or decrease ADFI (Sales, 2011). In the present studies, betaine supplementation did not affect litter performance, regardless of the season. These results are in agreement with Campbell and Virtanen (2011) who did not report an improvement in litter gain due to dietary betaine. Nonetheless, Ramis et al. (2011) and Greiner et al. (2014) reported an improvement in litter gain and no differences in the number of pigs weaned when betaine was supplemented.

Surprisingly, sows weaned in the non-summer presented signs of estrus 0.8 days later than sows weaned in the summer months. Supplementation of betaine during lactation consistently reduced weaning to estrus interval (Ramis et al., 2011; Greiner et al., 2014; Cabezon et al., 2016). In the summer experiment, betaine supplementation during lactation did not impact the number of days to estrus, however, betaine supplementation post-weaning reduced days to estrus. In the non-summer experiment, betaine supplementation during lactation reduced the number of days to estrus; however, betaine supplementation post-weaning did not affect days to estrus.

In the summer experiment, supplementation of betaine post-weaning reduced farrowing rate in P3+ sows. In the exploratory analysis, we observed a numeric reduction in farrowing rate (-11%) when P1 sows were fed betaine during lactation, and this effect was associated with a reduction in ADFI and greater BW losses. In the non-summer experiment, supplementation of betaine during lactation reduced farrowing rate in P3+ sows. This reduction was primarily driven by a greater culling rate due to reproductive reasons. Campbell and Virtanen (2001) reported a reduction in farrowing rate when P1 sows were fed betaine at 0.4%

(83% farrowing rate), but not when fed 0, 0.1 or 0.2% (89, 94 and 91 % farrowing rate, respectively). In contrast, other studies have not reported a decrease in farrowing rate due to betaine supplementation (Ramis et al., 2011; Greiner et al., 2014; Cabezon et al., 2016).

The greatest benefit of using betaine in diets of sows has been reported for subsequent litter size. Campbell and Virtanen (2001) reported that P2+ sows increased total number of pigs born by 2.3 piglets, while parity 1 did not show an increase. Van Wettere et al. (2012) reported an increase in subsequent litter size when betaine (7.6 to 9 g/sow/d) was fed to mature sows during gestation by 1.5 piglets more. We did not observe an increase in litter size when betaine was supplemented in the non-summer experiment. Nonetheless, in the summer experiment betaine supplementation during lactation to P4+ sows increased total number of pigs born by 1.2 piglets and supplementation of betaine in the post-weaning period increased total number of pigs born by 1.5 piglets.

The mechanism by which betaine may reduce the weaning to estrus interval and increase litter size is not yet fully understood. Recently studies in mice have shown a possible mechanism of betaine increasing embryo development and survival. Transporters of betaine are active during the 2 and 4-cell stage (approximately up to 30 hours after fertilization) (Anas et al., 2008). During these stages betaine accumulated in the rodent embryo and potentially served as an osmoprotectant prior to implantation in the initial cellular division and later as a methyl donor in the blastocyst (Corbett et al., 2014). The enzyme betaine-homocysteine methyltransferase has been thought to be unique to the liver (Finkelstein, 1990). Recent studies have shown that this pathway also exists in the blastocyst (Lee et al., 2012; Zhang et al., 2015).

Lee et al. (2012) found that impaired activity of betaine-homocysteine methyltransferase in the embryo causes embryo reabsorption.

Results of the present study suggest that the effects of betaine are primarily observed during the summer month. Supplementation of betaine during summer can improve sow reproductive performance by reducing the weaning to estrus interval and increasing subsequent litter size. However, supplementation of betaine had detrimental effects because it reduced ADFI and farrowing rate, and these two effects may be correlated. Further research needs to be conducted to determine if the detrimental effects are related to the level of inclusion.

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Table 1. Composition of the experimental diets, as fed basis[1].

	Diet[2]	
Item	Lactation	Post-weaning
Ingredient, %		
Corn, medium grind	45.92	47.03
Soybean meal, 46.5% CP	32.10	4.00
Rice bran	10.00	15.00
Wheat middlings	6.00	30.00
Poultry fat	2.35	0.00
Limestone	1.12	1.45
Monocalcium phosphate, 21% P	0.99	0.87
Potassium, magnesium sulfate[3]	0.50	0.50
Salt	0.40	0.40
Sow vitamin-mineral premix[4]	0.20	0.20
Choline chloride, 60%	0.13	0.13
Anti-caking aid[5]	0.10	0.10
Organic mineral source [Zn-Mn-Cu][6]	0.08	0.08
L-Lysine	0.05	0.15
L-Threonine	0.04	0.08
DL-Methionine	0.02	0.00
Iron oxide[7]	0.03	0.03
Calculated nutrient composition		
NRC ME Mcal/Kg	3.30	3.04
CP, %	21.17	12.90
Lysine, %	1.17	0.64
SID Lys, %	1.05	0.56
g SID Lys/Mcal ME	3.31	1.82
SID Met + Cys:Lys	0.56	0.69
Calcium, %	0.82	0.85
Available Phosphorous, %	0.4	0.44
Choline, g/kg	1.90	1.15

[1]Diets were formulated to exceed NRC (2012) requirements.
[2]To create betaine added diets, betaine (Vistbet®, AB Vista, Malborough, UK) was added at 0.2% at the expense of corn.
[3]Dynamate (Mosaic, Plymouth, MN), added as a laxative.
[4]Supplied per kg of complete diet: vitamin A, 11,023 IU; vitamin D_3, 1,763.7 IU; vitamin E, 51 IU; vitamin K, 4.4 mg; vitamin B12, 0.044 mg; riboflavin, 8.8 mg; d-pantothenate, 26.5 mg; niacin 55.1 mg; thiamine, 3.3 mg; pyridoxine, 3.3 mg; folic acid, 1.21 mg; biotin, 0.28 mg. Zn, 125 mg; Fe, 100 mg; Mn, 50 mg; Cu, 25.0 mg; I, 0.7 mg; Se, 0.3 mg; phytase, 661 FTU (Phyzyme, Danisco A/S, Copenhagen, Denmark), and chromium, 0.4 mg/kg.
[5]Dry anti-caking aid and non-nutritive carrier (KALLSIL, Kemin Industries, Inc., Des Moines, IA).
[6]Supplied per kg of complete diet: zinc, 50 mg from zinc amino acid complex, manganese, 20 mg from manganese amino acid complex, and copper, 10 mg from copper amino acid complex (Availa, Zimpro Corporation, Eden Prairie, MN).
[7]Used to color code diet. Diet with 0% betaine contained Fe_3O_4 (black) and diet with 0.2% betaine contained Fe_2O_3 (red).

Table 2. The analyzed nutrient composition of the experimental diets, as fed basis[1].

			Analyzed Composition, %			
Diet	Experiment[2]	Added Betaine, %	CP	Crude Fat	Crude Fiber	Ash
Lactation	1	0.0	22.09	5.68	3.10	7.16
	1	0.2	19.34	4.82	3.28	6.20
	2	0.0	21.66	5.86	3.08	6.91
	2	0.2	21.25	6.01	3.20	6.89
Post-weaning	1	0.0	12.51	5.00	4.19	7.16
	1	0.2	12.81	5.42	4.13	6.96
	2	0.0	13.23	4.93	4.36	6.65
	2	0.2	13.26	5.37	4.27	6.75

[1]Analyzed by the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO.

[2]Exp. 1 was conducted during summer months, and Exp. 2 was conducted during non-summer months.

Table 3. Effect of supplementation of betaine during lactation on sow and litter performance during summer months[1].

	Parity 1 and 2		Parity 3+				
	Betaine, %					P-values[2]	
Item	0	0.2	0	0.2	SEM	Betaine	Parity Group
Sows, n[1]	159	163	165	162			
BW at placement, kg[3]	246.4	247.2	278.9	280.9	2.143	0.443	<0.001
BW at farrowing, kg[4]	227.1	227.3	257.7	257.5	1.966	0.983	<0.001
BW at d 21, kg	209.1	206.2	251.8	249.2	1.898	0.148	<0.001
BW change, kg	-18.0	-21.0	-5.9	-8.2	1.399	0.024	<0.001
Sow ADG, kg/d	-0.856	-1.002	-0.283	-0.391	0.067	0.024	<0.001
Lactation length, d	22.10	22.09	22.16	22.22	0.101	0.748	0.245
ADFI, kg/d	3.687	3.414	4.873	4.823	0.094	0.052	<0.001
Sow G:F	0.428	0.388	0.475	0.464	0.022	0.224	0.004
Litter wt at placement, kg[5]	17.90	18.14	18.45	18.76	0.225	0.164	0.004
Litter wt at d 21, kg	68.9	67.2	71.5	72.0	0.879	0.431	<0.001
Litter gain, kg	52.7	51.1	54.8	55.2	0.727	0.350	<0.001
Pigs weaned per litter	11.02	10.94	10.92	10.90	0.097	0.535	0.432
Piglet mortality, %	8.08	8.73	8.92	9.15	0.804	0.548	0.385
No-value pigs, %	2.30	1.81	2.12	1.38	0.370	0.071	0.368

[1]Values represent least squares means of n sows for sow and litter performance. The Experiment was conducted from June to September.

[2]The statistical analysis tested for main effects of betaine supplementation, parity group and their interaction. Group of placement (1 to 28) was used as random effect. No significant interaction was found ($P \geq 0.13$).

[3]Sow BW was measured prior to sows entering the farrowing room (109 ±1 d of gestation).

[4]Sow BW after farrowing was calculated using the equation by Walker and Young (1991).

[5]Litters were standardized at 12 pigs per litter.

Table 4. Effect of betaine supplementation during lactation on rectal temperature and respiration rate in young (parity 1 and 2) and mature (parity 3 to 6) sows[1].

	Parity 1 and 2		Parity 3+			P-values[2]		
	Betaine, %							
Item	0	0.2	0	0.2	SEM	Betaine	Parity Group	Interaction
Summer[3]								
Rectal temperature, °C	39.70	39.36	39.53	39.57	0.119	0.211	0.876	0.122
Respiration rate[4]	65.77	62.58	81.90	74.89	8.684	0.562	0.110	0.827
Non-summer[5]								
Rectal temperature, °C	39.39	39.48	39.25	39.18	0.141	0.975	0.131	0.588
Respiration rate[4]	37.80	32.25	34.00	37.75	3.155	0.777	0.789	0.147

[1]Measurments were taken during the lactation period on d 18 of lactation, between 1600 h and 1800 h.
[2]The statistical analysis tested for main effects of dietary betaine, parity group, and their interaction. Sow was used as random effect.
[3]Values represent least squares means of 11 sows. During data collection average temperature of the farrowing room was 26.56 ±2°C
[4]Respiration rate was measured as the number of flank movements per 30 s.
[5]Values represent least squares means of 14 sows. During data collection average temperature of the farrowing room was 23.61 ±1°C

Table 5. Effect of supplementation of betaine in lactation, post-weaning, or both on subsequent reproductive performance of sows during summer months[1].

	Parity 1 and 2				Parity 3+				SEM	P-values[2]		
	Betaine in Lactation, %											
	0	0	0.2	0.2	0	0	0.2	0.2				
	Betaine in Post-weaning, %											
Item	0	0.2	0	0.2	0	0.2	0	0.2		Parity Group	Betaine Lactation	Betaine Post-weaning
Number of sows	76	83	83	80	85	80	80	82				
Days to estrus	6.73	6.17	7.94	5.79	5.82	5.64	6.24	5.43	0.674	0.068	0.587	0.054
Bred:Weaned[3]	0.974	1.000	0.940	0.913	0.988	0.975	1.000	0.988	0.018	0.015	0.059	0.606
Bred within 14 d:Weaned	0.868	0.928	0.807	0.863	0.918	0.913	0.900	0.939	0.034	0.037	0.227	0.127
Returns:Bred within 14 d[4]	0.136	0.169	0.119	0.087	0.038	0.123	0.097	0.169	0.038	0.434	0.959	0.145
Farrowed:Weaned[5]	0.816	0.771	0.759	0.763	0.906	0.775	0.875	0.744	0.044	0.126	0.308	0.016
Cull:Weaned	0.053	0.108	0.072	0.138	0.047	0.075	0.063	0.049	0.029	0.098	0.648	0.104
Total pigs born[6]	12.55^{b}	13.33^{ab}	13.39^{ab}	12.35^{b}	14.17^{a}	13.30^{ab}	14.46^{a}	14.00^{a}	0.462	0.001	0.511	0.224
Pigs born alive[7]	11.94^{bc}	12.75^{abc}	12.84^{ab}	11.56^{c}	13.05^{ab}	12.59^{abc}	13.20^{a}	13.00^{ab}	0.439	0.026	0.827	0.367
Still born pigs[8]	0.62	0.58	0.55	0.79	1.11	0.71	1.27	1.00	0.160	0.001	0.196	0.286
Mummies	0.129	0.172	0.159	0.180	0.117	0.129	0.214	0.164	0.061	0.927	0.327	0.880

[1]Values represent least squares means of n sows

[2]The statistical analysis tested for main effects of betaine supplementation during lactation, post-weaning, or both, parity group and their interaction. The group of placement (1 to 30) was used as the random effect in the analysis of days to estrus, total born, born alive, still born, and mummies.

[3]Lactation and parity group interaction ($P = 0.004$)

[4]Lactation and parity group interaction ($P = 0.058$).

[5] Sows that were bred within 14 days and farrowed. Post-weaning and parity group interaction ($P = 0.078$).

[6]Three factor interaction ($P = 0.087$).

[7]Three factor interaction ($P = 0.057$).

[8] Post-weaning and parity group interaction ($P = 0.051$).

[abc]Means with different superscripts differ ($P \leq 0.05$). Multiple comparisons used t-test.

Table 6. Exploratory analysis of the effect of dietary betaine in lactation, post-weaning, or both on subsequent reproductive performance during summer months[1]

	Betaine in Lactation, %					P-values		
	0	0	0.2	0.2				
	Betaine in Post-weaning, %							
Item	0	0.2	0	0.2	SEM	Betaine Lactation	Betaine Post-weaning	Interaction
Parity 1, n	39	44	44	42				
Days to estrus	5.78	6.82	7.80	5.19	0.960	0.843	0.415	0.061
Farrowed:Weaned[3]	0.795	0.750	0.682	0.810	0.066	0.687	0.533	0.195
Total pigs born	12.32[ab]	13.82[a]	13.40[ab]	12.18[b]	0.598	0.638	0.821	0.025
Pigs born alive	11.61[b]	13.33[a]	12.93[ab]	11.38[b]	0.598	0.599	0.888	0.007
Parity 2 and 3, n	71	71	71	72				
Days to estrus	7.21	5.69	7.16	6.66	0.815	0.569	0.216	0.526
Farrowed:Weaned[3]	0.859	0.803	0.859	0.736	0.046	0.469	0.052	0.469
Total born	13.42	13.20	13.41	13.01	0.541	0.848	0.550	0.864
Born alive	12.58	12.56	12.85	12.25	0.515	0.969	0.521	0.540
Parity 4, 5 and 6, n	51	48	48	48				
Days to estrus	5.28	5.56	6.25	4.51	0.685	0.957	0.242	0.107
Farrowed:Weaned[3]	0.922[a]	0.750[b]	0.875[ab]	0.729[b]	0.054	0.535	0.004	0.813
Total pigs born	14.15[ab]	12.90[b]	15.37[a]	14.02[ab]	0.585	0.026	0.014	0.927
Pigs born alive	13.07[ab]	12.06[b]	13.59[a]	12.88[ab]	0.552	0.178	0.084	0.768

[1]Values represent least squares means.

[2]The statistical analysis tested for main effects of betaine supplementation during lactation and/or post-weaning, and their interaction. The group of placement (1 to 30) was used as the random effect in the analysis of total born and born alive.

[3]Sows that were bred within 14 days and farrowed

[ab]Means with different superscript differ ($P \leq 0.05$). Multiple comparisons used t-test.

Table 7. Effect of supplementation of betaine during lactation on sow and litter performance during non-summer months[1].

	Parity 1 and 2		Parity 3+				
	Betaine, %					P-values[2]	
Item	0	0.2	0	0.2	SEM	Betaine	Parity Group
Sows, n	150	150	163	164			
BW at placement, kg[3]	225.8	223.6	276.7	275.7	2.337	0.469	<0.001
BW at farrowing, kg[4]	206.4	204.5	252.3	252.4	2.289	0.666	<0.001
BW at d 21, kg	199.5	196.5	255.0	254.2	2.245	0.348	<0.001
BW change, kg	-7.1	-7.9	2.0	1.8	1.428	0.698	<0.001
Sow ADG, kg/d	-0.340	-0.376	0.096	0.087	0.068	0.698	<0.001
Lactation length, d	22.96	23.18	23.16	23.27	0.113	0.075	0.110
ADFI, kg/d	4.31	4.24	5.61	5.47	0.101	0.155	<0.001
Sow G:F	0.412	0.401	0.444	0.486	0.015	0.203	<0.001
Litters, n[5]	81	84	93	94			
Litter wt at placement, kg[6]	17.21	17.10	19.40	19.29	0.287	0.687	<0.001
Litter wt at d 21, kg	62.0	60.7	69.6	69.9	1.304	0.699	<0.001
Litter gain, kg	45.3	43.6	50.2	50.7	1.253	0.622	<0.001
Pigs weaned per litter	10.79	10.76	10.78	10.72	0.100	0.687	0.816
Piglet mortality, %	10.10	10.27	10.14	10.62	0.837	0.704	0.816
No-value pigs, %	6.12	5.17	3.85	4.40	0.997	0.829	0.104

[1]Values represent least squares means This Experiment was conducted from February to June.

[2]The statistical analysis tested for main effects of betaine supplementation, parity group and the interaction, group of placement (1 to 28) was used as the random effect. No significant interaction was found ($P \geq 0.21$).

[3]Sow BW was measure previous to enter the farrowing room (112±2 d of gestation).

[4]Sow BW after farrowing was calculated using the equation by Walker and Young (1991).

[5]A subset of 352 sows was used to measure litter performance (group 1 to 16). Data was collected from February to April.

[6]Litters were standardized at 12 pigs per litter.

Table 8. Effect of supplementation of betaine on lactation and/or post-weaning on subsequent reproductive performance during non-summer months[1].

	Parity 1 and 2				Parity 3+					P-values[2]		
	Betaine in Lactation, %											
	0	0	0.2	0.2	0	0	0.2	0.2	SEM	Parity Group	Betaine Lactation	Betaine Post-weaning
	Betaine in Post-weaning, %											
Item	0	0.2	0	0.2	0	0.2	0	0.2				
Sows, n	77	73	74	76	81	82	83	81				
Days to estrus[3]	6.67[ab]	8.19[a]	6.83[ab]	5.76[b]	8.21[a]	6.93[ab]	7.26[ab]	6.70[ab]	0.752	0.401	0.077	0.473
Bred:Weaned[4]	0.987	0.918	0.973	0.921	1.000	1.000	0.988	0.963	0.019	0.005	0.268	0.008
Bred within 14 d:Weaned	0.831	0.753	0.838	0.829	0.778	0.817	0.855	0.790	0.044	0.930	0.290	0.369
Returns:Bred within 14 d[5]	0.109	0.109	0.081	0.095	0.143	0.119	0.239	0.219	0.044	0.008	0.214	0.809
Farrowed:Weaned[5,6]	0.818	0.726	0.851	0.750	0.864	0.854	0.711	0.716	0.046	0.995	0.073	0.128
Cull:Weaned[7]	0.104	0.153	0.108	0.171	0.025	0.061	0.120	0.148	0.035	0.069	0.040	0.079
Total born	12.54	12.37	13.53	12.19	14.49	13.77	14.00	13.66	0.441	<0.001	0.868	0.040
Born alive	12.13	11.85	12.56	11.72	13.54	12.76	13.05	12.88	0.412	0.001	0.954	0.075
Still born	0.41	0.53	0.84	0.47	0.88	0.91	0.83	0.78	0.126	0.001	0.620	0.448
Mummies	0.222	0.132	0.143	0.158	0.305	0.214	0.322	0.138	0.064	0.073	0.536	0.054

[1]Values represent least squares means.

[2]The statistical analysis tested for main effects of betaine supplementation during lactation and/or post-weaning, parity group and their interaction. The group of placement (1 to 28) was used as the random effect in the analysis of days to estrus, total born, born alive, still born, and mummies.

[3]Three way interaction ($P = 0.09$)

[4]Post-weaning and parity group interaction ($P = 0.079$).

[5]Lactation and parity group interaction ($P \leq 0.05$).

[6]Sows that were bred within 14 days and farrowed.

[7]Lactation and parity group interaction ($P = 0.108$).

[ab]Means with different superscript differ ($P \leq 0.05$). Multiple comparisons used t-test.

CHAPTER V:

General Discussion

Introduction

Previous experiments conducted in heat-stressed poultry (Farooqi et al., 2005) and rabbits (Hassan et al., 2011) motivated us to evaluate betaine in heat-stressed pigs. The experiments presented in the previous chapters were designed to study the effect of dietary betaine in swine exposed to heat stress. Our findings suggested that betaine in diets of heat-stressed growing and finishing pigs did not improve growth, which was in contrast to observations in poultry and rabbits. In sows however, dietary betaine fed during the summer increased the subsequent litter size. Betaine reduced feed intake in finishing pigs and lactating sows.

Nutritional practices that improve productivity under stressful conditionsin a particular species are commonly evaluated and adopted in other species to treat the same problem. However, differences in the gastrointestinal tract, body size, phase of growth, environment nutritional requirements, and other factors are reasons of not all of the nutritional practices can be transferred to other livestock animals.

The present chapter discusses and summarizes possible explanations of why betaine was not an effective strategy to alleviate heat stress in pigs. In addition, we aim to conduct an economic analysis of the use of betaine in sows to further address recommendations for the application of this tool.

Dietary betaine improved growth in heat stressed poultry and rabbits, but not growing pigs

Due to the differences in body size across species, we estimated the amount of betaine intake per kg of BW per day and the amount of of betaine intake per kg of metabolic body weight (MBW) per day used in the experiments conducted in heat-stressed poultry, rabbits, pigs and sows in our experiments, and a separate dose-response experiment conducted in growing pigs by Matthew et al. (2001) (Table 1). The intake of betaine per kg of BW and MBW was estimated by multiplying the percentage of betaine in the diet or water with the average daily intake of feed or water and divided by the average BW or $BW^{0.75}$ (average of the initial and final BW or $BW^{0.75}$ for the period betaine was fed). In addition, we calculated the proportion change in growth (BW or ADG) and feed intake of betaine-fed animals relative to animals fed the control (0% betaine) for each level of betaine reported in each of the experiments using the least square means (Table 1). We observed that effect of betaine supplementation was variable across species and small animals received a lower amount of betaine based on BW or MBW.

In order to study the relationship between dietary betaine level and the effect on performance across species, we evaluated the correlation among betaine intake/kg of BW/d, betaine intake/kg of MBW/d, the proportional change in BW gain, and the proportional change in feed intake (Table 2). Results suggested that the intake of betaine/kg of MBW/d can describe better ($P \leq 0.047$) the changes in gain and feed intake across species than the intake of betaine/kg of BW/d ($P \geq 0.431$). For that reason, we conducted linear regression to determine the relation of the intake of betaine per kg of MBW and the proportional change of

gain and feed intake (Figure 1). We evaluated linear, quadratic, and exponential (decay 3 parameters) models. The quadratic function showed the lowest AIC and was selected to discuss the relationship (Figure 1).

We observed that high levels of betaine intake reduced the growth of most of the animals, with the exception of rabbits. Rabbits progressively increased growth with further increments of betaine. The reduction in growth in most of the species can be explained by the reduction in feed intake, with the exception of ducks. Ducks increased growth even when feed intake was reduced due to betaine. Among animals, pigs and sows seem to be the most sensitive to the reduction in feed intake caused by betaine. This effect has been previously reported when Eklund et al. (2005) and Ratriyanto et al. (2009) summarized the effect of betaine on the performance of broilers and pigs. They reported that the reduction in feed intake was more frequently observed in pigs than in broilers. Physiological difference can contribute to the fact that pigs were more sensitive to reduce feed intake; however, there is also evidence that diet composition can contribute to this reduction in feed intake. Matthews et al. (1998) and Haydon et al. (1995) reported that an increase in the lysine:calorie ratio when betaine is added in the diets of pigs caused a reduction of feed intake. The variation in diet composition may be part of the reason why Sales (2011) in a meta-analysis reported that the effect of betaine in feed intake of pigs is variable, meaning that betaine can increase or reduce feed intake in pigs.

Further research is needed to understand the relation of diet composition and betaine supplementation in pigs. Understating this relationshipmay enable us to use betaine more effectively in diets of heat-stressed pigs as was the case for poultry and rabbits.

Economic Analysis

In chapter IV, we reported that supplementation of betaine during the summer to sows increased subsequent litter size. The effect of betaine across parity differed and for that reason we conducted an independent economic analysis for parity 1 (Table 3), parity 2 and 3 (Table 4), and parity 4, 5, and 6 (Table 5). The revenue and cost reported in tables 3 to 5 were estimated as marginal changes relative to the control treatment (0% betaine). The revenue and cost were calculated based on the least square means, when the effect (main or interactive) was significant ($P \geq 0.05$) or near significant ($0.05 \geq P \geq 0.1$). Otherwise, we used the overall average of the parity group. In addition, we corrected the total number of weaned pigs in the subsequent farrowing for farrowing rate, still born pigs, and pig survival. We assumed that piglet survival was 15% among all treatments for pigs that were born in the subsequent cycle. For the revenue of weanling pigs, we assumed 32 dollars per pig, which is the current market price. For the expenses due to feed, we assumed that between weaning and insemination sows consumed 2.70 kg/d, and from insemination until 35 days post-insemination sows consumed 2.05 kg/d. The cost of betaine was $9.90/kg (Vistabet ® AB Vista, Marlborough, Wiltshire, United Kingdom).

Among all parity groups feeding betaine during lactation or post-weaning cost less than $2.00 per sow, and feeding betaine in both periods cost approximately $3.50. Parity 1 sows had the greatest return of investment when supplemented with betaine during the post-weaning period. The second best was when parity 1 sows were fed betaine during the lactation period. Parity 2 and 3 did not have positive profits because subsequent litter size was not increased due to betaine. Parity 2 and 3 sows fed betaine during the lactation performed equally to the

control, showing a small negative loss due to expenses of feeding betaine. Parity 4, 5 and 6 had a positive profit only when betaine was fed during the lactation period.

Table 6 summarizes the profit (or loss) obtained due to feeding of betaine for all parity groups. The table shows that a maximum profit was obtained when parity 1 sows received betaine post-weaning and parity 4, 5, 6 received betaine in lactation, with a minimum cost of feeding betaine during the lactation to parity 2 and 3. The practice of feeding betaine only to certain parity groups will be applicable only if the farm segregates the herd by parity. A second alternative and more applicable to standard management is supplementation of betaine during lactation to the entire herd. Betaine during the lactation can provide revenues in parity 1, 4, 5, and 6, with a minimum cost of feeding betaine to parity 2 and 3 according to the present analysis.

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Table 1. The effect of betaine on growth and feed intake on heat-stressed animals.

Animal	Betaine level	Temperature, °C	Age/BW	Betaine intake, mg/kg BW/d[1]	Betaine intake, mg/kg MBW/d[1]	Betaine effect	Reference
Broilers[3]	0.1% in diet	35	21 to 35 d	107.0	110.9	↑ 6% ADG* ↑ 8% ADFI*	Farooqi et al., 2005
Broilers[4]	0.05% in water	32	32 to 42 d	126.7	144.6	↑ 5% BW gain* ↑ 1% ADFI	Sayed et al., 2011
	0.1% in water			252.0	269.0	↑ 1% BW gain ↓ 2% ADFI	
Broilers[3]	0.1% in diet	33	21 to 42 d	85.6	95.9	↑ 15% BW* ↑ 2% ADFI	Akhavan-Salamat et al., 2016
Ducks[4]	0.05% in diet	Summer	1 to 84 d	46.6	54.6	↑ 10% BW gain* ↓ 7 % ADFI*	Awad et al., 2014
	0.10% in diet			92.0	107.8	↑ 10% BW gain* ↓ 8 % ADFI*	
	0.15% in diet			122.0	146.1	↑ 14% BW gain** ↓ 12 % ADFI**	
Rabbits[3]	0.025% in diet	30	42 to 84 d	14.1	16.0	↑ 10% ADG ↑ 1% ADFI	Hassan et al., 2011
	0.050% in diet			28.4	32.2	↑ 12% ADG ↑ 1% ADFI	
	0.075% in diet			44.9	51.9	↑ 22% ADG* ↑ 13% ADFI*	
	0.10% in diet			58.8	68.6	↑ 30% ADG* ↑ 15% ADFI*	

[1]Betaine intake per kg of BW and MBW ($BW^{0.75}$) was estimated by multiplying the percentage of betaine in the diet or water with the average daily intake of feed or water and divided by the average BW or $BW^{0.75}$ (average of the initial and final BW or $BW^{0.75}$), respectively.
[2]Betaine effect was estimated as the proportional difference relative to the control group (no betaine) for growth (BW or ADG) and feed intake. The effects were assigned with * when $P \leq 0.01$ and ** $P \leq 0.05$ were reported by the respective author.
[3]Treatments were supplemented with Betafin®, Danisco A/S, Marlborough, Wiltshire, United Kingdom.
[4]The source of betaine was not specified.

Table 1. Continue

Animal	Betaine level	Temperature, °C	Age/BW	Betaine intake, mg/kg BW/d[1]	Betaine intake, mg/kg MBW/d[1]	Betaine effect[2]	Reference
Pigs[3]	0.10% in diet	31	45 to 64 kg	32.6	89.0	↑ 7% ADG ↑ 1% ADFI	Chapter II
	0.15% in diet			48.6	133.0	↑ 3% ADG ↑ 2% ADFI	
	0.20% in diet			66.1	181.3	↑ 3% ADG ↑ 4% ADFI	
Pigs[3]	0.2% in diet	31	91 to 122 kg	53.3	171.5	↓ 2% ADG** ↓ 4% ADFI**	Study I Chapter III
Pigs[3]	0.0625% in diet	33	96 to 120 kg	14.3	45.9	↓ 1% ADG 0% ADFI	Study II Chapter III
	0.1250% in diet			26.7	85.9	↓ 2% ADG ↓ 2% ADFI	
	0.1875% in diet			42.9	138.3	0% ADFI	
Pigs[3]	0.125% in diet	No heat stress	69 to 115 kg	38.3	119.4	↓ 4% ADG ↓ 10% ADFI*	Matthews et al., 2001
	0.250% in diet			74.5	232.0	↓ 8% ADG ↓ 12% ADFI*	
	0.500% in diet			154.3	480.9	↓ 8% ADG ↓ 9% ADFI*	
Lactating sows[4]	0.2% in diet	25	1 to 6 parity	35.0	137.2	↓ 8% ADG** (sow+litter) ↓4% ADFI**	Study I Chapter VI
Gestating sows[4]	0.2% in diet	24	1 to 6 parity	17.9	69.6	No recorded	
Lactating sows[4]	0.2% in diet	23	1 to 6 parity	42.8	166.0	↓ 2% ADG (sow+litter) ↓ 2% ADFI	Study II Chapter VI
Gestating sows[4]	0.2% in diet	24	1 to 6 parity	18.1	70.2	No recorded	

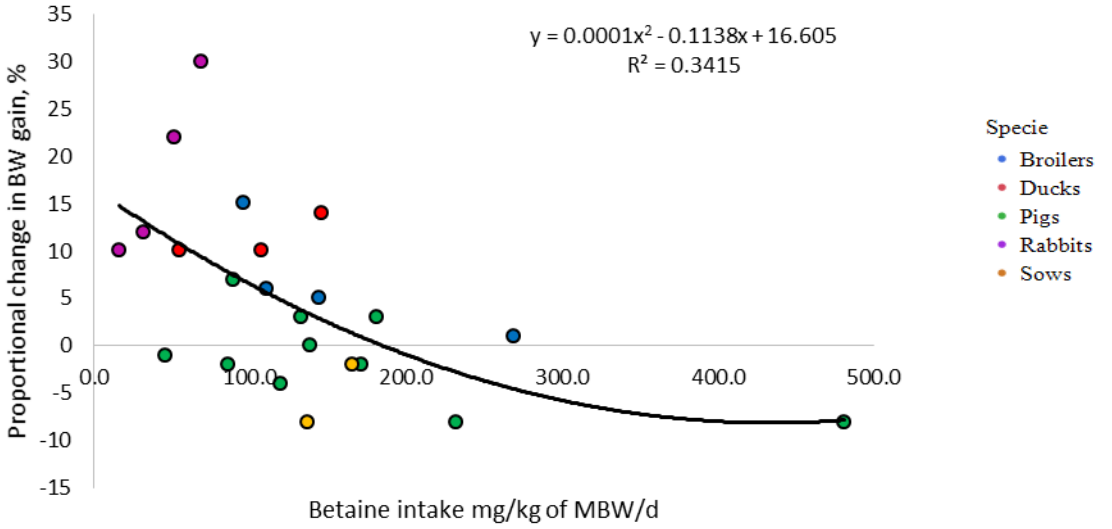
[1]Betaine intake per kg of BW and MBW ($BW^{0.75}$) was estimated by multiplying the percentage of betaine in the diet or water with the average daily intake of feed or water and divided by the average BW or $BW^{0.75}$ (average of the initial and final BW or $BW^{0.75}$), respectively.
[2]Betaine effect was estimated as the proportional difference relative to the control group (no betaine) for growth (BW or ADG) and feed intake. The effects were assigned with * when $P \leq 0.01$ and ** $P \leq 0.05$ as were reported by the respective author.
[3]Treatments were supplement with Betafin®, Danisco A/S, Marlborough, Wiltshire, United Kingdom.
[4]Treatments were supplement with Vistabet ® AB Vista, Marlborough, Wiltshire, United Kingdom

Table 2. Correlation matrix of betaine intake, growth, and feed intake of heat-stressed animals.

	Correlation Coefficients			
	P- value			
	Betaine intake/BW	Betaine intake/MBW[1]	Proportional change in gain[2]	Proportional change in feed intake[2]
Betaine intake/BW	1.000	0.651	-0.061	-0.173
		0.001	0.781	0.431
Betaine intake/MBW		1.000	-0.542	-0.418
			0.008	0.047
Proportional change in BW gain			1.000	0.617
				0.002
Proportional change in feed intake				1.000

[1]Betaine intake per kg of BW and MBW ($BW^{0.75}$) was estimated by multiplying the percentage of betaine in the diet or water with the average daily intake of feed or water and divided by the average BW or $BW^{0.75}$ (average of the initial and final BW or $BW^{0.75}$), respectively.
[2]The proportional difference relative to the control group (no betaine) for growth (BW gain or ADG) and feed intake.

A



B

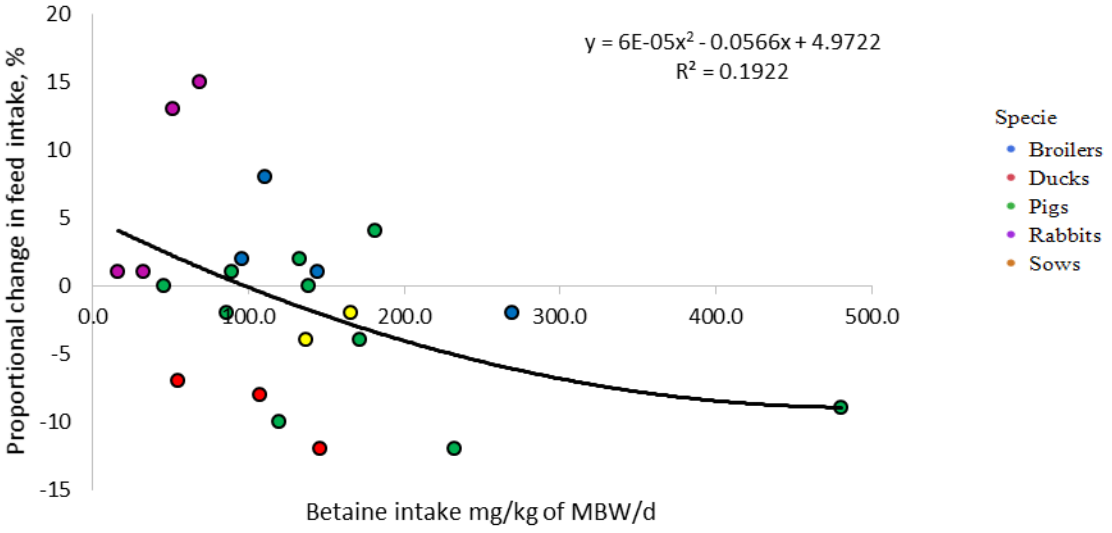


Figure 1. Relation between betaine intake per kg of MBW ($BW^{0.75}$) per day and proportional change in BW gain and feed intake across species. Proportional change was estimated relative to the control diet (0% betaine) in each of the experiments. Each circle represent the proportional change on **A.** gain and **B.** feed intake. Color blue, red, green, purple and yellow represent broilers, ducks, growing and finishing pigs, rabbits, and sows, respectively

Table 3. Economic analysis of betaine supplementation in Parity 1 sows.

	Betaine in Lactation, %			
	0	0	0.2	0.2
	Betaine in Post-weaning, %			
Item	0	0.2	0	0.2
Income				
Total pigs born, pigs[1]	12.3	13.8	13.4	12.2
Still born, %[2]	4.9	4.9	4.9	4.9
Farrowing rate, %[2]	75.9	75.9	75.9	75.9
Piglet survival, %[3]	85.0	85.0	85.0	85.0
Total weaned pig	7.6	8.5	8.2	7.5
Marginal weaned pig	0.00	0.92	0.66	-0.09
Marginal income for weaned pig, $/sow/summer[3]	0.00	29.46	21.21	-2.75
Cost				
Lactation feed intake, kg/d[4]	3.16	3.16	3.01	3.01
Lactation days, d[2]	22.1	22.1	22.1	22.1
Post weaning feed intake, kg/d[3]	2.70	2.70	2.70	2.70
Days to estrus[5]	6.79	6.00	6.79	6.00
Post insemination feed intake, kg/d[3]	2.05	2.05	2.05	2.05
Days post-insemination, d	35	35	35	35
Total betaine intake, kg	0.00	0.18	0.13	0.31
Dietary betaine cost, $/sow/summer[3]	0.00	1.74	1.32	3.06
Profit, $/sow/summer	0.00	27.71	19.89	-5.81

[1]Betaine during lactation and post-weaning interaction (P = 0.025).
[2]The general average across the four treatments (no significant effect).
[3]Assumptions: piglet mortality during the lactation 15%; market price of weanling pig $32; market price of a weanling; sows post-weaning eta 2.70 and 2.05 kg of feed before and after insemination, respectively; betaine market price was $ 9.9 per kg of product (Vistabet ® AB Vista, Marlborough, Wiltshire, United Kingdom).
[4]Supplementation of betaine during lactation reduced ADFI (P = 0.052).
[5]Supplemetnation of betaine post-weaning reduced days to estrus (P = 0.054).

Table 4. Economic analysis of betaine supplementation in parity 2 and 3 sows.

	Betaine in Lactation, %			
	0	0	0.2	0.2
	Betaine in Post-weaning, %			
Item	0	0.2	0	0.2
Income				
Total pigs born, pigs[1]	13.3	13.3	13.3	13.3
Still born, %[1]	5.3	5.3	5.3	5.3
Farrowing rate, %[2]	85.9	77.0	85.9	77.0
Piglet survival, %[3]	85.0	85.0	85.0	85.0
Total weaned pig	9.2	8.2	9.2	8.2
Marginal weaned pig	0.00	-0.96	0.00	-0.96
Marginal income for weaned pig, $/sow/summer	0.00	-30.64	0.00	-30.64
Cost				
Lactation feed intake, kg/d[4]	4.42	4.42	4.29	4.29
Lactation days, d	22.1	22.1	22.1	22.1
Post weaning feed intake, kg/d	2.70	2.70	2.70	2.70
Days to estrus[5]	7.25	6.16	7.25	6.16
Post insemination feed intake, kg/d	2.05	2.05	2.05	2.05
Days post-insemination, d	35	35	35	35
Total betaine intake, kg	0.00	0.18	0.19	0.37
Dietary betaine cost, $/sow/summer	0.00	1.73	1.88	3.67
Profit, $/sow/summer	0.00	-32.37	-1.88	-34.31

[1]The general average across the four treatments (no significant effect).
[2]Betaine during lactation post-weaning reduced farrowing rate ($P = 0.052$).
[3]Assumptions: piglet mortality during the lactation 15%; market price of weanling pig $32; market price of a weanling; sows post-weaning eta 2.70 and 2.05 kg of feed before and after insemination, respectively; betaine market price was $ 9.9 per kg of product (Vistabet ® AB Vista, Marlborough, Wiltshire, United Kingdom).
[4]Supplementation of betaine during lactation reduced ADFI ($P = 0.052$).
[5]Supplemetnation of betaine post-weaning reduced days to estrus ($P = 0.054$).

Table 5. Economic analysis of betaine supplementation in parity 4, 5 and 6 sows.

	Betaine in Lactation, %			
	0	0	0.2	0.2
	Betaine in Post-weaning, %			
Item	0	0.2	0	0.2
Income				
Total pigs born, pigs	14.2	12.9	15.4	14.0
Still born, %	9.8	9.8	9.8	9.8
Farrowing rate, %	89.9	74.0	89.9	74.0
Piglet survival, %	85.0	85.0	85.0	85.0
Total weaned pig	9.7	7.3	10.6	7.9
Marginal weaned pig	0.00	-2.43	0.84	-1.80
Marginal income for weaned pig, $/sow/summer	0.00	-77.88	26.89	-57.56
Cost				
Lactation feed intake, kg/d	5.06	5.06	4.89	4.89
Lactation days, d	22.1	22.1	22.1	22.1
Post weaning feed intake, kg/d	2.70	2.70	2.70	2.70
Days to estrus	5.72	4.92	5.72	4.92
Post insemination feed intake, kg/d	2.05	2.05	2.05	2.05
Days post-insemination, d	35	35	35	35
Total betaine intake, kg	0.00	0.17	0.22	0.39
Dietary betaine cost, $/sow/summer	0.00	1.73	1.88	3.67
Profit, $/sow/summer	0.00	-79.60	25.01	-61.22

[1]Main effect of betaine during lactation (P = 0.026) and post-weaning (P = 0.014).
[2]The general average across the four treatments (no significant effect).
[3]Assumptions: piglet mortality during the lactation 15%; market price of weanling pig $32; market price of a weanling; sows post-weaning eta 2.70 and 2.05 kg of feed before and after insemination, respectively; betaine market price was $ 9.9 per kg of product (Vistabet ® AB Vista, Marlborough, Wiltshire, United Kingdom).
[4]Supplementation of betaine during the lactation reduced ADFI (P = 0.052).
[5]Supplemetnation of betaine post-weaning reduced days to estrus (P = 0.054).

Table 6. Summary of the economic analysis of betaine supplementation during the summer to parity 1 to 6.

	Betaine in Lactation, %			
	0	0	0.2	0.2
	Betaine in Post-weaning, %			
Item	0	0.2	0	0.2
Parity 1				
Marginal income for weaned pig, $/sow/summer	0.00	29.46	21.21	-2.75
Feed cost, $/sow/summer	0.00	1.74	1.32	3.06
Profit, $/sow/summer	0.00	**27.71**	**19.89**	-5.81
Parity 2 and 3				
Marginal income for weaned pig, $/sow/summer	0.00	-30.64	0.00	-30.64
Feed cost, $/sow/summer	0.00	1.73	1.88	3.67
Profit, $/sow/summer	0.00	-32.37	-1.88	-34.31
Parity 4, 5, and 6				
Marginal income for weaned pig, $/sow/summer	0.00	-77.88	26.89	-57.56
Feed cost, $/sow/summer	0.00	1.73	1.88	3.67
Profit, $/sow/summer	0.00	-77.94	**25.01**	-59.56