EFFECT OF DIETARY L-CARNITINE ON FINISHING PIG GROWTH PERFORMANCE, MEAT QUALITY, AND STRESS PARAMETERS DURING HANDLING

by

BRADLEY WILLIAM JAMES

B.S., Iowa State University, 1999
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AN ABSTRACT OF A DISSERTATION

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Abstract

Four experiments were conducted to determine the interactive effects of dietary L-carnitine and ractopamine·HCl (ractopamine) on finishing pig growth performance. In analysis of treatments common to all experiments, ractopamine increased ($P < 0.01$) ADG and G:F compared to pigs not fed ractopamine. Added L-carnitine tended to increase ($P < 0.07$) ADG and improved ($P < 0.01$) G:F compared to pigs not fed L-carnitine. Three experiments were conducted to determine the effects of L-carnitine and ractopamine on carcass characteristics and meat quality. In Exp. 1, drip loss decreased (linear, $P < 0.04$) in pigs fed increasing L-carnitine. In Exp. 2, drip loss decreased ($P < 0.04$) with increasing L-carnitine when fed with ractopamine. Percentage lean was higher ($P < 0.01$) for pigs fed ractopamine. In Exp. 3, lean percentage increased ($P < 0.03$) in pigs fed L-carnitine or ractopamine. Pigs fed L-carnitine tended ($P < 0.06$) to have decreased drip loss. These results suggest that ractopamine increases carcass leanness and L-carnitine reduces drip loss when fed in combination with ractopamine. Two experiments were conducted to determine the effects of L-carnitine and ractopamine on the metabolic response to handling. Non-gentle handling increased ($P < 0.01$) lactate and rectal temperature, and decreased pH. In Exp. 1, non-gentle handled pigs fed ractopamine had decreased ($P < 0.01$) pH and increased temperature and tended ($P < 0.09$) to have higher lactate than other pigs. In Exp. 2, lactate and temperature changes from immediately post-handling to 1 h post-handling were not different for pigs fed L-carnitine or ractopamine suggesting that L-carnitine did not decrease recovery time of pigs subjected to non-gentle handling or fed ractopamine. These results suggest that pigs fed ractopamine are more susceptible to stress when handled aggressively. Because carnitine did not alleviate the negative effects of handling for pigs fed ractopamine, the improvement in drip loss from feeding carnitine must be due to a different mode of action.

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CHAPTER 1 - A Review of the Effects of Handling and Transportation on Stress in Swine

Abstract

Pigs are subjected to several stressors during typical production (Hyun et al., 1998). These stressors can negatively affect immune function (Hermann et al., 1993), growth performance (Sutherland et al., 2006), and meat quality (Hambrecht et al., 2005). Some of the stressors that affect pigs are heat stress, cold stress, social mixing, restricted floor space, facility design, handling intensity, loading and unloading from trailers, and transportation. These stressors often result in either transport losses or losses to the producer because of the impact on meat quality (Carr et al., 2005). The cost of pigs that die or become non-ambulatory during transportation or at packing plants translates to losses of about $100 million annually to the U.S. swine industry (Ellis et al., 2003). These losses have become a focus of attention for pork producers and packers alike. Recent research has concentrated on the effects of handling practices, seasonal or environmental variation, animal movement distances, trailer and facility design, and transportation distance. Potential methods to alleviate the stress induced to pigs during handling and transportation include: 1) walk pens prior to movement of pigs, 2) move pigs in small groups, 3) minimize use of electric prods, 4) load pigs during the cooler hours of the day, and 5) decrease the stocking density of pigs in trailers. Dietary supplementation of magnesium aspartate, L-tyrosine, vitamin E, vitamin D, vitamin C, and L-carnitine have all been evaluated as potential nutritional methods to decrease the stress affect on metabolic pathways and acid-base balance which also influence pork quality. The results of these studies have been variable. It appears that the best methods to reduce losses associated with stress are to improve handling and transportation practices.

Key Words: Handling, Stress, Transportation

Introduction

Stressors that trigger a physiological response can be acute/short-term (loud noise, unfamiliar environment, fighting, electric prod goading) or chronic/long-term (sickness, dehydration, malnourishment, heat/cold stress; Berg, 2001). The degree of stress an animal experiences in situations that are non-painful, such as transportation or handling, is directly determined by the degree of fear possessed during the event (Grandin, 1998).

Approximately 80,000 hogs per yr die during the transportation process: 70% of these losses occur on the truck during transit, and 30% occur shortly after arrival and are directly attributable to the transportation process (Grandin, 1992). Assuming an average market value of $100 per pig, these losses equate to an annual $8 million loss to the pork industry (Speer et al., 2001). More recently, Carr et al. (2005) reported that approximately 0.5 to 0.1% of pigs is characterized as downer pigs in commercial pork processing plants. The economic consequences of pigs that die or become non-ambulatory during transportation or at the packing plant cost about $100 million annually to the U. S. swine industry (Ellis et al., 2003).

In addition to losses due to stresses associated with transportation, stresses from improper handling have a negative affect on pork quality. The fight or flight response occurs when pigs are stressed and results in increased body temperature, blood pressure, and secretion of stress hormones such as cortisol and catecholamines. Consequential heat and energy are trapped in muscle that can lead to pale, soft and exudative pork (PSE) at slaughter, which tends to be tough, dry and less appetizing to the consumer (Berg, 2001). In 2002, approximately 15.5% of pork carcasses in the United States were considered PSE (Stetzer and McKeith, 2003). The Pork Chain Quality Audit estimated that PSE costs the pork industry nearly $30 million annually (Cannon et al., 1994).

This review will focus on the effects of transportation and handling stress as well as physical and nutritional methods to alleviate the effects of stress prior to slaughter.

Biology of Stress

The response to stressors requires a progression of events beginning with sensing and signaling an animal's various biological mechanisms that a threat exists (von Borell, 2001). Stress represents the body's reaction to stimuli that disturbs its normal physiological equilibrium

or homeostasis. One response to acute stressors is activation of the hypothalamic-pituitary-adrenal axis, resulting in elevated corticotropin-releasing hormone, which stimulates the anterior pituitary to release ACTH and other peptides (Hicks et al., 1998). Haussmann et al. (2000) observed that ACTH administration to sows caused a threefold increase in plasma cortisol concentrations. The effects of cortisol, a typical glucocorticoid, are best described as catabolic because cortisol promotes protein breakdown and decreases protein synthesis in skeletal muscle. Cortisol activity increases blood glucose concentrations by stimulating gluconeogenesis and creating a state of insulin resistance (Stockham and Scott, 2002).

Short stressful events (i.e., direct handling, isolation, and transportation) are usually followed by an increase in stress hormones, whereas chronic events (i.e., long-acting heat stress or restraint) might not affect the basal hormone concentration at all or even lower (down-regulation) the concentration in the blood (as reviewed by Ladewig, 2000).

Stressful events prior to slaughter can decrease pork quality. Postmortem metabolism of intramuscular energy stores (glycogen) plays the primary role in the conversion of muscle to meat and the expression of different attributes of fresh pork (NPPC, 2000). One of the most significant reactions is glycolysis, which results in the accumulation of lactic acid and reduces muscle pH. The development of acidic conditions causes denaturation of myofibrillar proteins and reduces water-holding capacity (Hembrecht et al., 2005).

Glycolysis is a process of anaerobic metabolism and results in the accumulation of lactate. In the living state, anaerobic metabolism is initiated when muscle is subjected to an oxygen shortage from situations such as intense exercise. Lactate is transported to the liver and converted to glucose. The process of anaerobic metabolism in animals also occurs following slaughter. As a result, changes occur in the concentrations of glycolytic substrates and reaction products until a point is reached at which some reaction in the glycolytic process is arrested and metabolism ceases (Faustman, 1994).

The color of meat usually is related to the muscle pH or its myoglobin content (Miller, 1994). Postmortem pH is one of the most important factors influencing drip loss (den Hertog-Meischke et al., 1997). The ability of muscle proteins to bind to water is decreased with low pH; however, that does not account for the total fluid loss. Those authors suggested that low pH reduced the negative electrostatic repulsion between filaments, thus diminishing the space between the filaments and causing shrinkage of myofibrils.

Transportation and Handling

Swine can be stressed and losses can occur resulting from numerous factors such as genetics, carcass muscling, health status, structural soundness, BW, nutrition, handling, facility design, and conditions during transport to the plant (Ritter et al., 2006). Animals can eventual become accustomed to various handling (Terlouw and Porcher, 2005) and husbandry practices leading to minimization of stress and fear; however, attempts to habituate animals for transportation are not practical and therefore some level of psychologically induced stress is inherently associated with livestock transportation (Speer et al., 2001). Geverink et al. (1998) observed that pigs that were allowed to move freely for 8 min outside their home pen, transported in a box for 2 min, and subjected to human contact during the finishing period were easier to load at slaughter; however, meat quality was not improved compared to pigs that were not handled during the finishing period. Grandin (1998) suggested that pigs will be easier to load into trucks and handle at the packing plant if producers walk through the pens frequently during finishing. The objective of walking the pens is to train the pigs to calmly get up and flow around the person, thus teaching the pigs to drive. However, spending more than 10 to 15 seconds per pig per day may cause the pigs to become too tame and difficult to drive (Grandin, 1998).

Loading

Physiological stress of livestock during transportation occurs as a result of the trucking process and the simultaneous deprivation of feed and water (Speer et al., 2001). Loading and off-loading have been found to be the most stressful parts of transportation, improving these processes reduce stress and trauma (Barton Gade, 1998). Research with sheep indicated that the most stressful part of transportation was the loading process and the initial period spent in the truck (Knowles et al., 1995). The authors suggested that the stress response decreases over time as livestock adjust and habituate to the transportation process in the truck on long hauls. Loading during the cooler hours of the day or during the night is helpful in reducing stress (Murray, 2000). The loading chute angle should not exceed a 20-degree incline for non-adjustable ramps and should not exceed a 25-degree incline for adjustable chutes (Grandin, 1988). A pig's heart rate will increase as the angle of a loading ramp increases (cited by Grandin, 1998)

Finishing pigs loaded into a trailer using electric prods had increased heart rate, rectal temperature, and levels of activity (rooting and investigative behavior) 15-min post-loading compared to pigs that were loaded with a hurdle (Brundige et al., 1998).

Weather conditions during transport also have an effect – hot weather leads to a greater PSE-frequency, while extremely cold weather leads to a greater energy consumption, and hence a higher DFD-frequency (Barton Gade, 1971, 1974).

Pigs transported under normal Spanish commercial conditions for 15 min and immediately slaughtered upon arrival to the packing plant had greater lactate and cortisol concentrations and resulted in lower longissimus thoracis pH measured 24 h postmortem compared to pigs that were transported for 3 h (Perez et al., 2002). The transportation time of 3 h may have allowed the pigs to adapt to the transport conditions and thus acted as a resting period like a lairage time. The short time between loading and unloading of pigs was likely a major contributor to the stress that affected lactate and cortisol. This study suggests that allowing pigs time to rest (lairage) after transportation may improve meat quality (decrease the response to stress) for pigs hauled short distances. Hambrecht et al. (2005) observed that pigs transported a short distance had increased cortisol when followed by short lairage and pigs transported a long distance tended to have increased glycolytic potential. Generally, the results indicate that lairage durations between 30 min and 3 h did not promote glycogen depletion or replenishment. However, suboptimal transport and lairage conditions exacerbated the effects of high preslaughter stress (stunning with electrical prods vs. calm handling), especially for those traits related to water-holding properties, such as increased plasma lactate, cortisol, and muscle temperature (Hambrecht et al., 2005). These results suggest that one stress alone may not clearly decrease pork quality, and likewise, improving one segment of transportation and handling may not improve meat quality. Transportation and handling stress is a unique non-steady-state situation and it is not likely that any single regimen will be totally effective in this environment as a treatment (Schaefer et al., 1997).

Pigs subjected to heat, cold, or transportation stress did not consistently have elevated glucocorticoids (plasma cortisol) even though other signs of acute stress were evident (Hicks et al., 1998). This suggests that pigs responded with different behaviors and indicators to various stressors and that no one measure was consistent across each stressor.

Methods of Alleviate Stress

Fasting

Pre-slaughter fasting improves food safety by decreasing stomach contents which lessens the potential for meat contamination. In addition, fasting lowers the glycogen content of muscles at slaughter and increases ultimate pH (Warriss and Brown, 1983), which may improve pork quality. Fasting pigs prior to slaughter reduces the amount of glucose stored as glycogen in the liver and muscle. Glycogen stored in the liver is more readily available for glycogenolysis because the enzyme glucose-6-phosphotase is present in the liver but not in muscle (Murray et al., 2000). Leheska et al. (2003) observed that pigs that were fasted for 48-h prior to slaughter had higher longissimus dorsi pH, darker-colored lean, and higher water-holding capacity than pigs that were not fasted. However, an interaction was observed for fasting (0 or 48-h) and transportation length (0.5, 2.5, or 8.0 h) where fasting improved semimembranosus pH, L*, color score, and drip loss for pigs that were transported 0.5 h, but when pigs were transported for 2.5 or 8.0 h, fasting had little or no effect on these muscle quality traits (Leheska et al., 2003). This implies that pigs that are transported using good-handling practices will deplete glycogen stores during extended periods of transportation compared to short hauling distances. Producers that are located near the packing plant may improve meat quality by fasting the pigs prior to transport; however, producers located further from the packing plant may not need a pre-slaughter fast. In contrast, Bertol et al. (2005) reported that pigs fasted for 24 h did not have improved blood lactate and pH values compared to pigs that were not fasted when subjected to high handling intensity.

Lawrence et al. (1998) observed that pigs that were subjected to a 24 h fast had induced erosion of the pars esophageal tissue, an indicator of early ulcer formation. This indicates that fasting is also a stress and should be avoided unless conducted prior to slaughter.

Stocking Density

Optimal stocking densities during transport are still a matter of debate but will vary depending on transport time, genotype and climate (Barton Gade, 1998). Pigs should have a minimum of 0.1 m^2 at 23 kg BW, 0.2 m^2 at 45 kg, 0.3 m^2 at 90 kg, 0.4 m^2 at 113 kg, and 0.6 m^2 at 181 kg during transport (Grandin, 1988; 1989). Ritter et al. (2006) observed a reduction in

transport losses as floor space on the trailer increased from 0.39 to 0.48 m^2/pig for pigs weighing 129 kg. Pigs should be provided with additional space in hot weather. Grandin (2001b) recommends that pigs weighing 90 kg and 113 kg BW should be provided with 0.33 m^2 and 0.46 m^2 , respectively, during transport when humidity is high and temperature exceeds 24° C This is 10 to 20% more space than would be recommended under normal conditions.

However, more space is not always better. According to a popular press article (Pork, 2003) the Danes videotaped and monitored the impact of transporting pigs with a greater space allowance. They found that the extra space caused pigs to fight more, be thrown around, and it increased skin damage, having a negative affect on pig welfare. Similarly, Barton-Gade and Christensen (1998) found that there were no benefits to additional space on short trips that were under 3 h during moderate weather.

The transportation distance and duration impacts the space needed to avoid additional stress. Market weight pigs remain standing when the trip is below 3 h and lie down on longer trips (Guise et al., 1998). For trips greater than 3 h, increase the space 15 to 20% depending on temperature (Grandin, 2001a). Decreasing the number of pigs on a trailer, or increasing the space, will allow pigs to lie down without being on top of each other.

If pigs are to be exposed to new or unfamiliar pigs from other pens, this should be done immediately before loading and transportation since pigs usually do not fight in a moving vehicle (Grandin, 2001b).

Grandin (1992) outlined several key components to minimize economic losses and stress which include: 1) During hot weather, livestock should be hauled at night or early morning in order to minimize the effects of heat stress. 2) Discourage commingling of strange animals to minimize the effects of behavioral stress and injuries that may result from fighting. 3) Shipment of wet animals during cold weather should be avoided to prevent deaths due to wind chill. 4) Livestock should be unloaded and rested if the trip will last more than 48 h. 5) Guidelines should be used for proper load density with respect to space requirements given various weather environments.

Prepare the animals

Grandin (2001a) suggested that the single most important issue is having an animal that is fit for transport. The author suggested that to improve fitness for transport, animals should be structurally sound and have correct feet and legs. The National Pork Board advises that pigs that are unable to walk or those that are ill and will not recover should be humanely euthanized on the farm and not transported to market channels (Grandin, 2002). Observations at two integrated pork companies where pig genetics is all the same have shown that excitability problems can be reduced and pigs will be easier to move at the plant if the producer walks through the finishing pens every day (Grandin, 2000).

Handling

Handling intensity had a major effect on blood acid-base balance immediately post-handling for pigs subjected to a high-intensity handling treatment (16 stimulations from electric prod) compared to a low intensity handling treatment (pigs allowed to move at their own pace) through a 98 m course (Hamilton et al., 2004). There was no affect of body weight (light, 104 kg; heavy, 128 kg) on baseline measurements of blood pH, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen, (PO_2), saturated oxygen (SO_2), total carbon dioxide (TCO_2), bicarbonate (HCO_2), base excess, and lactate. After handling, light pigs had higher blood SO_2, and showed a greater increase in PO_2 from baseline to post-handling than heavy pigs which indicate that the light pigs absorbed more oxygen and/or consumed less oxygen during handling than heavy pigs. This suggests a limited impact of live weight across the range evaluated on the response to handling.

Ritter et al. (2009) subjected pigs to 3 concurrent stressors: 1) handling intensity (gentle vs. aggressive), 2) transport floor space (0.39 vs 0.49 m^2/pig), and 3) distance moved during handling (25 vs. 125 m) to determine the effects on metabolic responses. The effects of the stressors were additive on rectal temperature, blood acid-base balance, and LM lactate. The authors suggested that removing just 1 stressor during the marketing process may improve acid-base balance and decrease the incidence of fatigued pigs.

Stress during handling can result in the condition of metabolic acidosis that is characterized by an increase in blood lactate and decreased in blood pH (Ellis, 2003; Peterson et al., 2009). Characteristic symptoms of this condition are open-mouthed breathing, cyanosis, and

unresponsiveness to stimuli to move, i.e., the characteristic symptoms of a non-ambulatory (downer) pig.

Muscle and Genetics

Modern hybrid pigs, which have been selected for rapid growth, leanness, and large loin area, are often prone to stress that causes the pig to become nonambulatory (Grandin, 2001a). Pigs that are carriers for the halothane gene (HAL) have a much higher incidence of PSE meat (De Smet et al., 1996). The HAL gene causes an overflow of calcium ions across the sarcoplasmic reticulum and extends the stimulation of muscle contraction, and thus increases glycogen metabolism and heat production (Dikeman, 2003). Pigs that are HAL carriers are more susceptible to stressors such as transportation and handling prior to slaughter and have increased pH decline and elevated body temperature which result in poor pork quality.

The Napole gene (RN) results in increased glycogen concentrations and thus high glycolytic potential in the live animal that cause low ultimate pH and subsequent poor pork quality (Sellier and Monin, 1994). The low ultimate pH causes decreased water holding capacity or increased drip loss. Because of their negative impacts on meat quality, the halothane and napole genes have been removed from most of the domestic pig population.

Facilities

Well-designed facilities and trucks will facilitate animal loading and unloading and minimize the stress on the animals (Ellis, 2003). Stress can be reduced by placing cleats on loading chutes or having properly spaced steps. Cleats should be 2.5 cm × 2.5 cm and placed 20 cm apart on center for loading market weight pigs. Missing or damaged cleats should be repaired to prevent leg injuries. Steps should have a 6.5 cm rise and a 25 cm long tread (Grandin, 2002). The loading chute ramp angle should not exceed 20 degrees for a non-adjustable ramp and 25 degrees for an adjustable ramp. It is also important to have non-slip flooring on loading ramps and alley floors (Grandin, 2002). Facility design should allow pigs to follow each other rather than squeeze together at gates and chutes. Pigs will often move up a ramp more easily if they are moved outside of the building before they encounter the ramp. Pig movement through alleys and chutes is greatly affected by air movement, shadows and lighting. Pigs have a tendency to move from a darker area towards a brighter area, but will not approach a blinding light (Grandin, 2002).

Potential Nutritional Affects

D'Souza et al. (1998) observed that pigs fed magnesium aspartate for 5 d prior to slaughter had lower norepinephrine concentrations at slaughter compared to pigs fed the control diet. The authors also observed that pigs fed magnesium aspartate had lower muscle lactic acid (longissimus thoracis and biceps femoris) and suggested that this was because of reduced effects of stress on muscle glycogenolysis which in turn resulted in higher muscle pH, decreased percentage drip loss, and less pale meat compared to pigs fed the control diet. However, Caine et al. (2000) observed mixed meat quality responses to pigs fed a magnesium aspartate and the responses were dependent upon level and duration of supplementation. Magnesium supplementation may slightly improve electrolyte balance but the major contribution may be the affect on animals' response and resistance to stress by altering the release of stress hormones (Classen et al., 1987). Supplemental magnesium had no affect on stress responses of finishing pigs measured after 3 h of transportation; however, 0 and 45-min muscle pH was higher for pigs fed supplemental magnesium although pork quality was not improved (Apple et al, 2005).

Amino acid therapy may affect meat quality in any of three ways: providing amino acids as physiological regulators of stress (substrates for neurotransmitters), as substrates for protein synthesis, or as substrates for gluconeogenesis (Schaefer et al., 2001). Supplementing a swine finishing diet with 10 g of L-tyrosine/kg for 5 d before slaughter significantly increased the concentrations of plasma tyrosine, hypothalamic tyrosine, and various catecholamine metabolites in the hypothalamus (Adeola and Ball, 1992). Tyrosine is a nonessential large neutral amino acid and is the precursor of the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine (Schaefer et al., 2001). Thorough investigations of tyrosine supplementation have not been conducted likely due to the economic cost of supplementation. Similar to tyrosine, evidence for a significant impact of tryptophan on pork quality is not conclusive. Supplementation of tryptophan for 5 d before slaughter doubled plasma tryptophan and increased hypothalamic serotonin 44%; however, muscle pH, color, and other meat quality attributes were not affected. Roberts et al. (1996) observed that tryptophan supplementation modified catecholamine flux, but also had no affect on pork quality.

Vitamin E is an antioxidant and may improve meat quality by inhibiting the conversion of oxymyoglobin (red color) to metmyoglobin (brown color), or by maintaining cell membrane

stability, which reduces drip loss and oxidative rancidity (Pettigrew and Esnaola, 2000). Vitamin D supplementation has also been evaluated as a method to improve meat quality. Studies have shown that feeding high concentrations of vitamin D improve beef tenderness by increasing plasma and muscle calcium concentrations that stimulates activity of calpains that have been shown to enhance meat tenderness. However, in pigs the results have been variable (Enright et al., 1998; Wiegand et al., 2002).

Ascorbic acid is required for synthesis of cortisol and is depleted during stress (Schaefer et al., 2001). Vitamin C can be metabolized into oxalic acid, which has been shown to inhibit glycolysis and in turn improve pork quality (Kremer et al., 1998). Inhibiting glycolysis may reduce the amount of lactate that is produced and thus reduce the rate/level of pH decline, which in turn would improve water-holding capacity and color. Pantothenic acid is also required for synthesis of cortisol and is depleted during stress (Schaefer et al., 2001). Studies have shown that increased supplementation of panthothenic acid may increase longissimus muscle area and decrease fat depth (Stahly and Lutz, 2001). Groesbeck et al. (2004) fed pigs increased levels of panthothenic acid and observed no improvement in pork quality.

Supplementing pigs with L-carnitine and ractopamine HCl in combination have been shown to decrease drip loss and improve meat quality (James et al., 2003). Carnitine has been shown to increase pyruvate carboxylase and decrease lactate dehydrogenase, which may reduce substrate for lactic acid synthesis postmortem. Bertol et al. (2005) reported that supplementing L-carnitine had a relatively small but positive effect on decreasing blood pH changes in finishing pigs submitted to handling stress. However, blood metabolites that can influence blood pH such as lactate, HCO_3^-, PO_2, and PCO_2 were not significantly affected during handling by L-carnitine supplementation.

Similar to carnitine, supplementing pigs with creatine may reduce drip loss (James et al., 2002). Creatine provides high-energy phosphate for the conversion of ADP to ATP and it is speculated that creatine may delay postmortem glycolysis and delay the associated drop in pH.

Other compounds that have been studied as possible influences on pork quality include supplemental conjugated linoleic acid, betain, selenium-enriched yeast, and chromium. However, results from these studies have been variable and it is difficult to draw clear conclusions on their impact.

Handling and transportation are stressors that pigs encounter during typical production. These stressors negatively impact immune function, growth performance, and meat quality. An understanding of the biology of these stressors will enable the discovery of methods to help alleviate the negative impact. It is important that producers have a clear understanding of proper handling and transportation procedures and that facilities are well designed and maintained to reduce the amount of stress a pig receives.

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CHAPTER 2 - Interactive Effects Between Dietary L-carnitine and Ractopamine-HC1 on Finishing Pig Performance: I Growth Performance

Abstract

A total of 2,152 pigs were used in four experiments to determine the interactive effects of dietary L-carnitine and ractopamine·HCl (RAC) on finishing pig growth performance. All trials were arranged as factorials with main effects of L-carnitine (0, 25, or 50 ppm in Exp. 1 and 2 and 0 or 50 ppm in Exp. 3 and 4) and RAC (0, 5, or 10 ppm in Exp. 1 and 0 or 10 ppm in Exp. 2, 3, and 4). Dietary carnitine was fed from 38 to 109 kg (Exp. 1 and 3) or for the last 4 or 3 wk before slaughter (118 kg; Exp. 2 and 4, respectively). Ractopamine HCl was fed for 4 wk before slaughter in Exp. 1, 2, and 3, and 3 wk in Exp. 4. Experiments 1 and 2 were conducted in university research facilities and Exp. 3 and 4 in commercial research barns. All diets were formulated to 1.00% total lysine during the last phase of each experiment. In all experiments, pigs fed RAC had increased ($P < 0.05$) ADG and G:F compared to pigs not fed RAC. Feeding L-carnitine before the RAC feeding period did not affect ($P > 0.25$) pig performance. In Exp. 1 and 2, L-carnitine did not affect ($P > 0.46$) ADG during the last 4 wk; however, in Exp. 2, G:F tended (quadratic; $P < 0.07$) to improve with increasing L-carnitine. In Exp. 3, an L-carnitine × RAC interaction was observed ($P < 0.04$) for ADG and G:F. Both added L-carnitine and RAC improved performance, but the response was not additive. In Exp. 4, pigs fed L-carnitine had increased ($P < 0.04$) ADG (0.88 vs. 0.84 kg) and G:F (0.36 vs. 0.35) compared to pigs not fed L-carnitine and the response was additive to that of RAC. In analysis of the treatments common to all experiments, RAC increased ($P < 0.01$) ADG (1.03 vs. 0.93 kg) and G:F (0.40 vs. 0.35) compared to pigs not fed RAC. Added L-carnitine tended to increase ($P < 0.07$) ADG (1.00 vs. 0.96 kg) and improved ($P < 0.01$) G:F (0.38 vs. 0.37) compared to pigs not fed L-carnitine. These results confirm that RAC improves growth performance of finishing pigs. Added L-carnitine improved growth performance of finishing pigs with the greatest response observed in Exp. 3 and 4, which were conducted in commercial research environments.

Key words: L-carnitine, Pigs, Ractopamine HCl

Introduction

Research has shown that ractopamine HCl (RAC) improves growth performance and carcass leanness in pigs by directing nutrients away from fat deposition and towards protein deposition. Anderson et al. (1987) demonstrated that RAC enhances protein accretion and increases N retention. Bergen et al. (1989) also observed that pigs fed RAC had decreased protein degradation.

To support increased protein deposition, pigs fed RAC need a higher dietary lysine (protein) level than pigs not fed RAC (Dunshea et al., 1993, Webster 2007). Depending on feed intake and the increase in protein deposition, pigs fed RAC may be in an energy dependent phase of growth, despite energy available from decreased lipogenisis (Trapp et al., 2002; Apple et al., 2008).

Carnitine has an important role in intermediary energy metabolism. Owen et al. (2001a) observed pigs fed added L-carnitine were leaner and had decreased backfat thickness than those fed a control diet. The increased protein accretion and reduced backfat thickness were associated with greater rates of palmitate oxidation and more rapid flux through pyruvate carboxylase. Owen et al. (2001a) speculated that pigs fed added L-carnitine were able to use fat for energy and divert carbon towards synthesis of amino acids. Carnitine supplementation has been shown to stimulate pyruvate dehydrogenase complex by reducing the acetyl-CoA:CoA ratio in mitochondria via exporting the acetyl groups out of the mictochondria matrix (Xi et at., 2008). Therefore, we hypothesized that adding L-carnitine to the diet could increase the amount of energy available for protein deposition and increase the response to RAC. Therefore, the objective of these experiments was to determine the interactive effects among RAC and L-carnitine.

Materials and Methods

General

Procedures used in these experiments were approved by the Kansas State University Animal Care and Use Committee. The pigs were also part of a project evaluating the interactive effects of dietary L-carnitine and RAC on finishing pig carcass characteristics and meat quality (James et al., 2009). Experiments 1 and 2 were conducted at the Kansas State University Swine

Teaching and Research Center and Exp. 3 and 4 were conducted at a commercial research facility in southwestern MN. All pigs used in these experiments were progeny of C22 sows × 336 boars (PIC, USA, Hendersonville, TN). In Exp. 1 and 2, pigs were housed in an environmentally controlled building with 1.2 m × 1.2 m slatted-floor pens. Each pen had a one-hole self-feeder and a nipple waterer to allow ad libitum access to feed and water. In Exp. 3 and 4, pigs were housed in a curtain-sided barn with a deep pit and completely slatted floors. The barn was 12.5 × 76.2 m with 48, 3.05 × 5.49 m pens. Each pen contained one 4-hole dry self-feeder and 1 cup waterer to allow ad libitum access to feed and water. Ractopamine HCl was fed for 4 wk before the end of the study in Exp. 1, 2, and 3, and for 3 wk in Exp. 4. Dietary L-carnitine was fed from approximately 38 kg BW until the end of the study (Exp. 1 and 3) or for the last 4 or 3 wk before the end of the study (Exp. 2 and 4, respectively). At the end of Exp. 1, 2, and 3, pigs were slaughtered to evaluate carcass characteristics and meat quality. All dietary nutrients were formulated to meet or exceed the recommended requirement estimates (NRC, 1998).

Experiment 1

One hundred twenty-six gilts (initially 33.4 kg) were allotted by weight and ancestry in a randomized complete block design to 1 of 9 experimental treatments arranged in a 3 × 3 factorial. There were 2 pigs per pen and 7 pens (replicates) per treatment.

Pigs were fed a corn-soybean meal diet (Table 1) with added L-carnitine (0, 25, or 50 ppm) from 33.4 kg until the end of the experiment (approximately 109 kg). The basal diet was formulated to contain 1.10% total lysine (18.2% CP) from 33.4 to 74.4 kg, and 1.00% total lysine (16.9% CP) from 74.4 kg until the end of the study. Dietary RAC treatments (0, 5, or 10 ppm) were fed for the last 4 wk of the experiment.

Weights were obtained on all pigs and feeders every 14-d during the experiment until the last 4 wk, at which time measurements were recorded weekly to calculate ADG, ADFI, and G:F.

Experiment 2

One hundred twenty gilts (initially 87.2 kg) were allotted by weight and ancestry in a randomized complete block to 1 of 6 experimental treatments arranged in a 2 × 3 factorial. There were 2 pigs per pen and 10 pens per treatment. Pigs were fed a 1.00% lysine (16.9% CP) corn-soybean meal basal diet (Table 1) with added L-carnitine (0, 25, or 50 ppm) and RAC (0 or

10 ppm) for the 4-wk experiment. Weights were obtained on all pigs and feeders every 7 d during the experiment to calculate ADG, ADFI, and G:F.

Experiment 3

One thousand one hundred and four barrows (initially 44.0 kg) were allotted by weight in a randomized complete block to 1 of 8 experimental treatments arranged in a 2 × 2 × 2 factorial. There were 23 pigs per pen and 6 pens per treatment. The main effects included dietary L-carnitine (0 or 50 ppm), RAC (0 or 10 ppm), and added fat (0 or 6%). We speculated that the diets with added fat might give an indication as to the degree of energy sparing activity of L-carnitine or if it would respond additively or interactively with RAC.

Pigs were fed a corn-soybean meal diet (Table 1) with or without added L-carnitine and with or without added fat from 44.0 kg until slaughter (approximately 118 kg). Dietary RAC treatments (0 or 10 ppm) were fed for the last 4 wk of the experiment. The basal diet was formulated on a total lysine:calorie ratio basis with ratios of 3.16 g Lys/Mcal from 44.0 to 61.2 kg, 2.70 g Lys/Mcal from 61.2 to 92.0 kg, and 3.00 g Lys/Mcal from 92.0 kg until the end of the experiment. The corresponding total lysine levels in the 0 and 6% added fat diets were 1.05 and 1.14% (17.6 and 18.5% CP); 0.90 and 0.97% (15.6 and 16.3% CP), and 1.00 and 1.08% (17.0 and 17.8% CP) total lysine for the three phases, respectively. Weights were obtained on pens of pigs and feeders every 14-d during the experiment until the last 4 wk, at which time measurements were taken weekly to calculate ADG, ADFI, and G:F.

Experiment 4

Seven hundred ninety-six barrows (initially 103.0 kg) were allotted by weight in a randomized complete block to 1 of 4 experimental treatments arranged in a 2 × 2 factorial. There were 18 or 19 pigs per pen and 10 pens per treatment. The main effects included dietary L-carnitine (0 and 50 ppm) and RAC (0 and 10 ppm).

Pigs were fed a corn-soybean meal diet (Table 1) with or without L-carnitine or RAC for the 3-wk experiment. The basal diet was formulated to contain 1.00% total lysine (total lysine:calorie ratio of 3.00 g Lys/Mcal; 17.0% CP). Weights were obtained on pens of pigs and feeders weekly to calculate ADG, ADFI, and G:F.

Statistical analyses

Data from all experiments were analyzed as a randomized complete block design with pen as the experimental unit. Pigs were blocked based on initial weight at the beginning of each experiment, and analysis of variance was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Linear and quadratic polynomial contrasts were performed to determine the effects of carnitine level (Exp. 1) and RAC level (Exp. 1 and 2). Growth performance data from common treatments of L-carnitine (0 or 50 ppm) and RAC (0 or 10 ppm) from the 4 experiments were combined and analyzed for main effects and interactions between L-carnitine and RAC.

Results

Experiment 1

Supplementing finishing diets with L-carnitine did not affect ($P > 0.64$) growth performance of pigs between 33.4 and 74.4 kg (Table 2). Pigs were allotted to treatments at the initiation of feeding carnitine and remained within the same treatment groups for the duration of the experiment. This explains the numeric ($P < 0.22$) differences in initial weight at the beginning of the RAC feeding period (Table 3). Two pens within the same treatment (50 ppm L-carnitine and 5 ppm RAC) were removed from the experiment because of clinical ileitis; therefore values reported in the tables are means of 7 or 5 replications. There were no RAC × carnitine interactions ($P > 0.12$) observed for ADG, ADFI, or G:F during the last 4 wk of the experiment. Increasing RAC increased (quadratic, $P < 0.02$) ADG and improved (quadratic, $P < 0.01$) G:F. Average daily gain of pigs fed 5 ppm RAC was similar to control pigs, but ADG increased and was greatest for pigs fed 10 ppm RAC. Feed efficiency improved with increasing RAC and was highest for pigs fed 10 ppm RAC. Pigs fed 25 ppm L-carnitine had decreased (quadratic, $P < 0.06$) ADG and ADFI compared to pigs fed 0 or 50 ppm.

Experiment 2

There were no RAC× L-carnitine interactions observed ($P > 0.36$) for ADG, ADFI, or G:F (Table 4). Feeding pigs RAC improved ($P < 0.01$) ADG and G:F. There was no effect ($P > 0.46$) of feeding L-carnitine on ADG. However, pigs fed L-carnitine tended (quadratic, $P < 0.07$)

to have improved G:F, which was greatest for those pigs fed 25 ppm L-carnitine. The G:F response was due to a linear ($P < 0.01$) reduction in ADFI.

Experiment 3

There were no L-carnitine × RAC × fat interactions ($P > 0.10$) observed during the entire experiment (Table 5). There were no L-carnitine × fat interactions ($P > 0.73$) observed for growth performance of pigs between 44.0 and 92 kg (pre-RAC period). During this period, supplementing finishing pig diets with L-carnitine did not affect ($P > 0.25$) growth performance. As expected, addition of 6% dietary fat improved ($P < 0.01$) ADG and G:F and reduced ($P < 0.01$) ADFI during this period.

For the overall RAC supplementation period, there were no 3-way interactions ($P > 0.81$) or L-carnitine × fat interactions ($P > 0.21$). However, an L-carnitine × RAC interaction was observed ($P < 0.04$) for ADG and G:F. Added L-carnitine and RAC both improved ADG and G:F; however, the responses were not additive. Dietary fat decreased ($P < 0.01$) ADFI and improved G:F ($P < 0.05$).

Experiment 4

There were no L-carnitine × RAC interactions ($P > 0.47$) for growth performance for the overall experiment (Table 6). Overall, ADG and G:F was improved by adding RAC and L-carnitine to the diet for the last 3 wk before slaughter (118 kg). In this study, the response appeared to be additive, particularly for ADG.

Combined Growth Performance

The growth performance data from common treatments of L-carnitine (0 or 50 ppm) and RAC (0 or 10 ppm) from the 4 experiments were combined (Table 7). There were no L-carnitine × RAC interactions ($P > 0.27$) observed. Feeding pigs RAC improved ($P < 0.01$) ADG and G:F in these experiments. A numerical trend was observed for increased ADG ($P < 0.07$) when pigs were fed L-carnitine compared to those pigs fed the control diet. Pigs fed L-carnitine in the last 3 to 4 wk before slaughter also had improved ($P < 0.01$) G:F compared to pigs not fed L-carnitine. These results suggest that L-carnitine and RAC improve growth performance of finishing pigs.

Discussion

The improved ADG and G:F to added RAC observed in our studies were not surprising and were similar to other observations on the effect of feeding RAC to pigs (Armstrong et al., 2004; See et al., 2004 Apple et al., 2008). In young pigs, research evaluating the effects of L-carnitine on growth performance has been variable. Hoffman et al. (1993) and Cho et al. (1999) observed no improvements in growth performance when supplementing 800 to 1,000 ppm L-carnitine to diets for 21-d old pigs. However, Owen et al. (1996) reported that pigs supplemented with 500 ppm L-carnitine had a 9% improvement in feed efficiency. Rincker et al. (2003) demonstrated that weanling pigs fed 50 to 100 ppm L-carnitine had increased G:F approximately 2 to 4 wk after weaning compared to pigs not fed L-carnitine and suggested that the response to added L-carnitine may depend on dietary fat inclusion level.

In Exp. 1, added L-carnitine did not affect growth performance. These results are similar to those reported by Smith et al. (1996) who observed no difference in growth performance of pigs supplemented with 50 ppm L-carnitine from 36 to 66 kg, and 66 to 109 kg; however, pigs fed L-carnitine had numerically greater ADG compared to pigs fed the control diet. Similar to Exp. 1, there was no effect of feeding L-carnitine on ADG in Exp. 2. However, pigs fed L-carnitine tended to have increased G:F compared to pigs not fed L-carnitine with the greatest response for pigs fed 25 ppm L-carnitine. The major differences between Exp. 2 and Exp. 1 are: 1) only RAC 0 or 10 ppm RAC were fed compared to (0, 5, and 10 ppms fed in the Exp. 1; and 2) L-carnitine was only fed for 4 wk compared to approximately 6 wk. Feeding RAC improved both ADG and G:F in both experiments.

In Exp. 3, the responses we observed to added fat were consistent with previous findings (De La Llata et al. 2001, Webster et al., 2003) where finishing pigs reared in a commercial facility were fed diets with 6% added fat. We observed no interactions when feeding either RAC or L-carnitine with fat. The lack of an interaction with energy level is similar to the response observed by Trapp et al. (2002). Pigs fed either RAC or L-carnitine during the last 4 wk of the experiment had improved growth performance compared to pigs not fed RAC or L-carnitine, however, the response was not additive.

In Exp. 4, pigs fed L-carnitine had increased ADG from d 0 to 14 and for the length of the experiment (initially, 103 kg). Furthermore, pigs fed L-carnitine had numerically higher G:F during wk 2 and 3 of the experiment which resulted in an overall increase in G:F during the experiment compared to pigs not fed L-carnitine. Trapp et al. (2002) reported a similar benefit to adding L-carnitine to diets containing RAC; however, the greatest response occurred during the first two wk of their four-wk feeding period. In our experiment, ADG and G:F was improved due to adding RAC and L-carnitine to the diet for the last 3 wk before slaughter (118 kg) with the response being additive in this experiment. In our previous trial conducted in a commercial finishing facility, the response was not additive.

The marked improvement in ADG and G:F of pigs fed L-carnitine in the late finisher has not previously been well documented. A notable difference between our experiments with L-carnitine and RAC in the finisher and previous studies conducted by other researchers is that these pigs were fed a higher total lysine level than what would typically be fed in the late finishing period. This was done to assure adequate lysine for pigs consuming RAC which need a higher level of lysine to meet their protein deposition needs. Therefore, one might theorize that a higher level of lysine is also needed for protein deposition to demonstrate a growth response to feeding supplemental L-carnitine. Heo et al. (2000b) fed young pigs (17.8 kg) 500 ppm L-carnitine and either low (13.6% CP; 0.90% total lysine) or high (18.0% CP; 1.20% total lysine) protein diets. In their experiment pigs fed supplemental L-carnitine had increased ADG compared to pigs not fed L-carnitine and the response was numerically higher for pigs fed the high protein diet compared to those fed the low protein diet; however, no significant interaction (protein level × L-carnitine) was observed. The authors suspected that pigs fed the low protein diet were restricted sufficiently in daily ME for L-carnitine to affect both the low and high protein treatments. Owen et al. (2001a) observed that supplemental L-carnitine promoted fatty acid oxidation through accelerated β-oxidation, decreased flux of mitochondrial branched-chain keto acid dehydrogenase activity, and increased flux through pyruvate carboxylase. The authors suggested that these results explain that pigs fed L-carnitine are more able to use fat for energy, divert carbon toward synthesis of amino acids, and spare branched-chain amino acids for protein synthesis. This does not explain the response observed in our experiments because all pigs were fed diets containing at least 1.00% total lysine compared to the suggested requirement of 0.52% total lysine (80 to 120 kg; NRC, 1998) during the last phase of finishing. Therefore, it is unlikely

that the improvement in growth performance of pigs fed supplemental L-carnitine was an effect of L-carnitine sparing lysine or other amino acids for protein deposition.

A difference between Exp. 3 and 4 and others is that these pigs were reared in a commercial finishing facility. Feed intake is typically lower at commercial facilities compared to university facilities due to environmental and space allowance differences (De La Llata et al., 2002). In our experiments, feed intake for pigs reared at the commercial research facility and not fed added fat (Exp. 3 and 4) was lower (2.55 kg/d) compared with feed intake (2.70 kg/d) for pigs reared at the university research facility (Exp. 1 and 2), and the response to added L-carnitine was best for pigs reared at the commercial research facility (Exp 2 and 3) compared to pigs reared at the university research facility (Exp. 1 and 2). Heo et al. (2000b) fed young pigs (17.8 kg) a restricted diet (85% ad libitum) with or without added L-carnitine (0 or 500 ppm) and observed that feeding L-carnitine reduced urinary N excretion by 14% and improved biological value (defined as the percentage of absorbed N retained in the body) by 3% compared to pigs not fed L-carnitine. The authors suggested that the improved biological value of N in the L-carnitine supplemented pigs resulted in more dietary amino acids being used for body protein synthesis rather than for energy. This theory is supported by Owen et al. (2001a). It is plausible that in our experiments, pigs reared in the commercial research facility were in an energy depended phase of growth and not capable of maximizing protein deposition. Because of L-carnitine's effect on intermediary energy metabolism, pigs fed L-carnitine may have had more energy available for protein synthesis although the mode of action was not likely to be from sparing lysine because of the high levels that were fed to pigs in these experiments. Another difference between our experiments and others is that previous studies have not specifically examined the last four weeks per se (Smith et al., 1996; Owen et al., 2001a; Owen et al., 2001b). There may be some metabolic changes that are occurring as the pig becomes heavier which are affected by L-carnitine supplementation such as insulin resistance.

These studies demonstrate that supplemental L-carnitine and/or RAC improve growth performance of pigs in the last three to four wk of the finishing phase. Pigs reared in a commercial finishing facility had the greatest improvement in growth performance when fed L-carnitine compared to pigs reared at the university research facility. This may be a result of lower feed intake that occurs at commercial facilities compared to university research facilities due to environmental and space allowance differences. Care should be given when interpreting these

results because pigs in our studies were fed higher levels of lysine compared to what is typically fed to pigs in the late finisher. The growth performance response of pigs fed L-carnitine may be dependent on a higher level of lysine to meet the needs for increased protein deposition.

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Table 2.1 Basal diet composition (as-fed basis)[a]

	Exp. 1[b]		Exp. 2[c]	Exp. 3[d]						Exp.4[d]
Initial BW, kg:	33	74	87	44		61		92		103
Ingredient, %				No fat	Fat	No fat	Fat	No fat	Fat	
Corn	68.41	74.50	74.50	73.00	63.30	78.60	69.35	75.10	65.55	75.10
Soybean meal, 46.5 % CP	26.63	22.80	22.80	24.60	28.25	19.15	22.35	22.75	26.25	22.75
Choice white grease	-	-	-	-	6.00	-	6.00	-	6.00	-
Soybean oil	2.00	-	-	-	-	-	-	-	-	-
Monocalcium phosphate, 21% P	1.05	0.90	0.90	0.85	0.94	0.73	0.80	0.64	0.70	0.64
Limestone	1.00	0.90	0.90	0.88	0.84	0.85	0.83	0.84	0.81	0.84
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[e]	0.15	0.15	0.15	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Trace mineral premix[f]	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine·HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Medication[g]	0.05	-	-	-	-	-	-	-	-	-
DL-methionine	0.01	-	-	-	-	-	-	-	-	-
Cornstarch	0.05	0.10	0.10	-	-	-	-	-	-	-
Calculated composition										
CP (N × 6.25), %	18.20	16.90	16.90	17.60	18.50	15.60	16.30	17.00	17.80	17.00
Lysine, %	1.10	1.00	1.00	1.05	1.14	0.90	0.97	1.00	1.08	1.00
Lysine:calorie ratio, g/mcal	3.24	3.01	3.01	3.16	3.16	2.70	2.70	3.00	3.00	3.00
ME, kcal/kg	3,399	3,318	3,318	3,327	3,596	3,336	3,605	3,338	3,607	3,338
Ca, %	0.69	0.61	0.61	0.60	0.61	0.55	0.56	0.54	0.55	0.54
P, %	0.60	0.55	0.55	0.55	0.57	0.50	0.52	0.50	0.51	0.50

[a] Diets were formulated to meet or exceed NRC (1998) requirements.

[b] L-carnitine replaced cornstarch to provide either 0, 25, or 50 ppm L-carnitine. RAC replaced cornstarch to provide either 0, 5, or 10 ppm ractopamine·HCl.

[c] L-carnitine replaced cornstarch to provide starch either 0, 25, or 50 ppm L-carnitine. RAC replaced cornstarch to provide 0 or 10 ppm ractopamine·HCl.

[d] L-carnitine replaced corn to provide either 0, or 50 ppm L-carnitine and RAC replaced corn to provide 0 or 10 ppm ractopamine·HCl.

[e] In Exp. 1 and 2, vitamin premix provided (per kilogram of complete diet): vitamin A, 6,614 IU; vitamin D_3, 992 IU; vitamin E, 26.5 IU; menadione (menadione dimethylpyrimidinol bisulphite), 2.65; vitamin B_{12}, 0.03 mg; riboflavin, 5.95 mg; pantothenic acid, 19.8 mg; and niacin, 33.1 mg. In Exp. 3 and 4, vitamin premix provided (per kilogram of complete diet): vitamin A, 7,937 USP; vitamin D_3, 1,190 USP; vitamin E, 31.75 IU, vitamin B_{12}, 0.03 mg; riboflavin, 7.14 mg; pantothenic acid, 23.81 mg; niacin, 39.68 mg.

[f] In Exp. 1 and 2,, trace mineral premix provided (per kilogram of complete diet): Mn (from manganese oxide), 39.7 mg; Fe (from ferrous sulfate), 165.3 mg; Zn (from zinc oxide) 165.3 mg; Cu (from copper sulfate), 16.5 mg; I (from calcium iodate) 0.3 mg; and Se (from sodium selenite), 0.3 mg. In Exp. 3 and 4, trace mineral premix provided (per kilogram of complete diet): Mn (from manganese oxide), 35.7 mg; Fe (from ferrous sulfate), 148.8 mg; Zn (from zinc oxide), 148.8 mg; Cu (from copper sulfate), 14.9 mg ; I (from calcium iodate) 0.3 mg; and Se (from sodium selenite) 0.3 mg.

[g] Provided 44 mg tyloslin per kg diet.

Table 2.2 Effect of L-carnitine on growth performance of the finishing pig prior to feeding Ractopamine HCl (Exp. 1)[a]

	L-carnitine, ppm				Probability ($P <$)		
Item	0	25	50	SED	L-carnitine	Linear	Quadratic
ADG, kg	0.90	0.92	0.92	0.02	0.64	0.37	0.76
ADFI, kg	2.00	2.01	2.01	0.03	0.90	0.65	0.91
G:F	0.45	0.46	0.46	0.01	0.66	0.49	0.55

[a] Values represent the period from 33.4 to 74.4 kg BW. At 74.4 kg, pigs were switched to diets containing 0, 5, or 10 ppm Ractopamine HCl in addition to the L-carnitine levels. Values are means of 21 replications (pens) and 2 pigs per pen.

Table 2.3 Effect of L-carnitine and Ractopamine HCl on finishing pig growth performance (Exp. 1)[a,b]

	Ractopamine HCl, ppm											Probability ($P <$)					
	0			5			10										
	L-carnitine, ppm										Ractopamine HCl ×	Ractopamine		Ractopamine HCl		L-carnitine	
Item	0	25	50	0	25	50	0	25	50	SED	L-carnitine	HCl	L-carnitine	Linear	Quad[c]	Linear	Quad[b]
Initial wt	72.5	74.3	74.3	74.8	75.4	75.5	75.6	74.1	74.8	1.35	0.58	0.22	0.72	0.11	0.56	0.43	0.87
ADG, kg	0.97	0.99	1.09	1.05	0.99	0.97	1.12	1.01	1.10	0.04	0.12	0.07	0.13	0.88	0.02	0.73	0.04
ADFI, kg	2.49	2.50	2.67	2.49	2.40	2.41	2.66	2.38	2.50	0.08	0.17	0.21	0.16	0.08	0.77	0.84	0.06
G:F	0.39	0.40	0.41	0.42	0.41	0.40	0.42	0.42	0.44	0.01	0.53	0.01	0.55	0.05	0.01	0.32	0.64
Final wt	98.7	101.9	104.7	104.2	103.0	102.7	107.1	102.4	105.6	2.04	0.10	0.09	0.39	0.26	0.06	0.42	0.25

[a]Values are means of 7 or 5 replications (pens) and 2 pigs per pen for 28 d.

[a]Average initial BW, 74.4 kg.

[c]Quad (quadratic).

Table 2.4 Effect of L-carnitine and Ractopamine HCl on growth performance of the finishing pig (Exp. 2)[a]

	Ractopamine HCl, ppm											
	0			10				Probability ($P <$)				
	L-carnitine, ppm							Ractopamine HCl ×			L-carnitine	
Item	0	25	50	0	25	50	SED	L-carnitine	Ractopamine HCl	L-carnitine	Linear	Quadratic
Day 0 to 28												
ADG, kg	1.04	1.05	1.01	1.12	1.16	1.11	0.03	0.90	0.01	0.46	0.50	0.30
ADFI, kg	3.03	2.90	2.84	2.96	2.82	2.83	0.09	0.90	0.44	0.10	0.05	0.41
G:F	0.35	0.36	0.36	0.38	0.41	0.40	0.01	0.87	0.01	0.10	0.23	0.07

[a] Average initial BW, 87.2 kg.

[b] Values are means of 10 replications (pens) and 1 or 2 pigs per pen.

Table 2.5 Interactive effects of L-carnitine, Ractopamine HCl, and fat on growth performance of finishing pigs (Exp. 3)[a]

	Fat, %															
	0				6											
	L-carnitine, ppm															
	0		50		0		50									
	Ractopamine HCl, ppm									Probability (P <)						
Item	0	10	0	10	0	10	0	10	SED	Carn[b]* Ractopamine HCl *Fat	Carn[b]* Ractopamine HCl	Carn[b]* Fat	Ractopamine HCl *Fat	Carn[b]	Ractopamine HCl	Fat
Pre-Ractopamine HCl [c,d]																
ADG, kg	0.94	0.91	0.93	0.94	0.98	0.99	0.99	0.99	0.02	-	-	0.97	-	0.37	-	0.01
ADFI, kg	2.52	2.46	2.47	2.51	2.46	2.40	2.44	2.42	0.03	-	-	0.97	-	0.98	-	0.01
G:F	0.37	0.37	0.38	0.38	0.40	0.41	0.40	0.41	0.01	-	-	0.73	-	0.25	-	0.01
Day 0 to 28																
ADG, kg	0.83	0.94	0.93	0.97	0.87	0.98	0.93	0.96	0.02	0.82	0.04	0.21	0.92	0.02	0.01	0.46
ADFI, kg	2.67	2.63	2.72	2.69	2.59	2.54	2.58	2.51	0.04	0.86	0.91	0.21	0.71	0.55	0.14	0.01
G:F	0.31	0.36	0.34	0.36	0.34	0.38	0.36	0.38	0.01	0.81	0.01	0.53	1.0	0.01	0.01	0.05

[a]Values are means of six replications (pens) and 22 to 26 pigs per pen.
[b]Carn (L-carnitine)
[c]Initial BW of pre-ractopamine HCl period, 44.0 kg.
[d]Growth performance for pre-ractopamine HCl period was determined for d 0 to 51 prior to initiation of ractopamine HCl.
[e]Average BW at initiation of ractopamine HCl supplementation, 92.1 kg.

Table 2.6 Interactive effects of L-carnitine and Ractopamine HCl on commercial finishing pig growth performance (Exp. 4)[a,b]

	Ractopamine HCl, ppm							
	0		10					
	L-carnitine, ppm					Probability (P <)		
Item	0	50	0	50	SED	L-carnitine × Ractopamine HCl	L-carnitine	Ractopamine HCl
Day 0 to 21								
ADG, kg	0.76	0.81	0.91	0.96	0.02	0.94	0.01	0.01
ADFI, kg	2.39	2.43	2.39	2.45	0.04	0.80	0.15	0.81
G:F	0.32	0.38	0.33	0.39	0.01	0.47	0.04	0.01

[a]Values are means of 10 replications (pens) and 18 or 19 pigs per pen.
[b]Initial BW, 103.0 kg.

Table 2.7 Summary of the interactive effects of L-carnitine and Ractopamine HCl on finishing pig growth performance (Pooled data from Exps. 1, 2, 3, & 4)[a]

	Ractopamine HCl, ppm							
	0		10					
	L-carnitine, ppm					Probability (P <)		
Item	0	50	0	50	SED	L-carnitine x Ractopamine HCl	L-carnitine	Ractopamine HCl
ADG, kg	0.90	0.95	1.03	1.04	0.02	0.27	0.07	0.01
ADFI, kg	2.65	2.66	2.66	2.62	0.05	0.60	0.61	0.73
G:F	0.34	0.39	0.36	0.40	0.01	0.40	0.01	0.01

[a]Initial BW, 74.4, 87.2, 92.1, and 103.0 kg for Exp. 1, 2, 3, and 4, respectively. Values are means of 33 replications from 4 different experiments with 2, 2, 22 to 26, and 18 to 19 pigs per pen in experiment 1, 2, 3, and 4, respectively. Treatment diets were fed for 28 d in Exp. 1, 2, and 3 and for 21 d in Exp. 4.

CHAPTER 3 - Interactive Effects Between Dietary L-carnitine and Ractopamine·HCl on Finishing Pig Performance: II Carcass Characteristics and Meat Quality

Abstract

Three experiments utilizing 1,356 pigs were conducted to determine the interactive effects of dietary L-carnitine and ractopamine·HCl (RAC) on carcass characteristics and meat quality. Experiments were arranged as factorials with main effects of L-carnitine and RAC. Levels of L-carnitine were 0, 25, or 50 ppm in Exp. 1 and 2 and 0 or 50 ppm in Exp. 3. Ractopamine HCl levels were 0, 5, or 10 ppm in Exp. 1 and 0 or 10 ppm in Exp. 2, and 3. Dietary L-carnitine was fed from 38 kg to slaughter (109 and 118 kg, in Exp. 1 and 3, respectively) or for 4 wk before slaughter (109 kg, Exp. 2). Ractopamine HCl was fed for 4 wk. Experiments 1 and 2 were conducted at university research facilities (2 pigs per pen) and Exp. 3 in a commercial research barn (25 pigs per pen). An L-carnitine × RAC interaction ($P < 0.02$) was observed for visual color, L*, and a*/b* in Exp. 1. In pigs fed RAC, increasing L-carnitine decreased L* and increased visual color scores and a*/b* compared to pigs not fed RAC. Ultimate pH tended to increase (linear, $P < 0.07$) with increasing L-carnitine. Drip loss decreased (linear, $P < 0.04$) in pigs fed increasing L-carnitine. In Exp. 2, an L-carnitine × RAC interaction was observed ($P < 0.04$) for visual firmness and drip loss. Visual firmness scores decreased in pigs fed increasing L-carnitine and no RAC, but increased with increasing L-carnitine when RAC was added to the diet. Drip loss decreased with increasing levels of L-carnitine when fed with RAC. Percentage lean was higher ($P < 0.01$) for pigs fed RAC. An L-carnitine × RAC interaction ($P < 0.03$) was observed in Exp. 3 for fat thickness and percentage lean. Fat thickness decreased and lean percentage increased in pigs fed L-carnitine or RAC, but the responses were not additive. Pigs fed L-carnitine tended ($P < 0.06$) to have decreased drip loss. Pigs fed RAC had decreased ($P < 0.05$) 10^{th} rib and average backfat and decreased drip loss compared to pigs not fed RAC. These results suggest that RAC increases carcass leanness and supplemental L-carnitine reduces drip loss when fed in combination with RAC.

Key words: Carnitine, Pigs, Ractopamine HCl

Introduction

Ractopamine HCl (RAC) is a β-agonist that increases protein accretion (Anderson et al., 1987) and decreases protein degradation (Bergen et al., 1989). Cromwell (1991) reported that pigs fed ractopamine HCl (RAC) require additional ME for maintenance and increased the protein synthesis; however, this is offset by the reduced energy required because of decreased fat deposition. Because of low feed intake observed in commercial environments (De la Llata et al., 2001), pigs fed RAC may be in an energy dependent phase of growth, and thus, not capable of maximizing protein deposition. Apple (2008) observed that pigs fed RAC had increased carcass weight, longissimus muscle depth, and lean muscle yield and that carcass measures where similar between pigs fed RAC and either 5% beef tallow or soybean oil.

Heo et al. (2000a) demonstrated that carnitine is essential for intermediary energy metabolism. A major function of carnitine is to transport long chain fatty acid groups across the mitochondrial membrane into the mitochondrial matrix for energy production (adenosine triphosphate) via β-oxidation and oxidative phosphorylation. Adding L-carnitine to the diet could increase the amount of energy available for protein deposition and increase the response to RAC. Added L-carnitine has also been shown to influence the enzymes involved in lactic acid production. Owen et al. (2001a) demonstrated that supplementing L-carnitine increased pyruvate carboxylase and decreased lactate dehydrogenase flux in pigs. A reduction in post-mortem lactic acid production would increase pH and therefore might improve meat quality.

Therefore, the objectives of these experiments were to evaluate the interactive effects among L-carnitine, RAC, and dietary energy density on carcass parameters of growing-finishing pigs and to evaluate differences in longissimus quality indicators, such as color, marbling, and firmness.

Materials and Methods

General

Procedures used in these experiments were approved by the Kansas State University Animal Care and Use Committee. The pigs used in these experiments were also part of a project evaluating the interactive effects of dietary L-carnitine and RAC on growth performance of growing-finishing pigs (James et al., 2009). Experiments 1 and 2 were conducted at the Kansas State University Swine Teaching Research Unit and Exp. 3 was conducted at a commercial research facility in southwestern MN. All pigs used in these experiments were progeny of C22 sows × 336 boars (PIC, USA, Hendersonville, TN). Pigs were slaughtered and carcass and meat quality measurements obtained at the Kansas State University Meats Laboratory in Exp. 1 and 2, and at a commercial slaughter facility (Swift, Inc., Worthington, MN) in Exp. 3.

In Exp. 1 and 2, pigs were housed in an environmentally controlled building with 1.2 m × 1.2 m slatted-floor pens. Each pen had a one-hole self-feeder and a nipple waterer to allow ad libitum access to feed and water. In Exp. 3, pigs were housed in a curtain-sided barn with a deep pit and completely slatted floors. The barn was 12.5 × 76.2 m with 48, 3.05 × 5.49 m pens. Each pen contained one 4-hole dry self-feeder and one cup waterer to allow ad libitum access to feed and water. Ractopamine HCl was fed for 4 wk before the end of each experiment. Dietary L-carnitine was fed from approximately 38 kg BW until the end of the study (109 and 118 kg, Exp. 1 and 3, respectively) or 4 wk before the end of the study (109 kg, Exp. 2). All dietary nutrients were formulated to meet or exceed the recommended requirement estimates (NRC, 1998). All diets during the RAC feeding period contained at least 1.00% total lysine to account for the increase in protein deposition observed in pigs fed RAC (Webster et al., 2007).

Experiment 1

One hundred twenty-six gilts (initially 33.4 kg) were allotted by weight and ancestry in a randomized complete block to each of the nine experimental treatments arranged in a 3 × 3 factorial. There were 2 pigs per pen and 7 replicates per treatment.

Pigs were fed a corn-soybean meal diet (Table 1) with added L-carnitine (0, 25, or 50 ppm) from 33.4 kg until slaughter (approximately 109 kg). The basal diet was formulated to contain 1.10% total lysine (18.2% CP) from 33.4 to 74.4 kg, and 1.00% total lysine (16.9% CP)

from 74.4 kg until the end of the experiment (109 kg). Dietary RAC treatments (0, 5, or 10 ppm) were fed for the last 4 wk of the experiment.

One pig (closest to 109 kg) per pen was selected and slaughtered at the Kansas State University Meats Laboratory. Standard carcass measurements, visual analyses of longissimus muscle color (1 = pale pinkish gray to white, 2 = grayish pink, 3 = reddish pink, 4 = dark reddish pink, 5 = purplish pink, 6 = dark purplish red; NPPC, 2000), marbling (1 = 1% intramuscular fat, 2 = 2% intramuscular fat, 3 = 3% intramuscular fat, 4 = 4% intramuscular fat, 5 = 5% intramuscular fat, 6 = 6% intramuscular fat; NPPC, 2000), and firmness (1= soft, cut surface distorts easily, 2 = firm, cut surface tends to hold shape, 3 = very firm, cut surface very smooth and no distortion of shape; NPPC, 2000), color spectrophotometry (L*, a*, and b*; CIE, 1976), percentage drip loss (modified from Kauffman et al., 1986), ultimate pH, and temperature were obtained from each pig at 24-h postmortem with an Accument Portable pH Probe Model AP61 (Fischer Scientific, Pittsburgh, PA.

Experiment 2

One hundred twenty gilts (initially 87.2 kg) were allotted by weight and ancestry in a randomized complete block to each of the 6 experimental treatments arranged in a 2 × 3 factorial. There were 2 pigs per pen and 10 replicates per treatment. Pigs were fed a (1.00% lysine; 16.9% CP) corn-soybean meal basal diet (Table 1) with added L-carnitine (0, 25, or 50 ppm) and RAC (0 or 10 ppm) for the 4-wk experiment.

One pig (closest to 109 kg) per pen was randomly selected and slaughtered at the Kansas State University Meats Laboratory. Blood was collected as soon as possible after exsanguination and pH, glucose, and lactate were measured from whole blood. Longissimus muscle pH and temperature were measured as soon as possible after exsanguination and at 15 min, 45 min, 1.5, 3, and 6 h postmortem. Standard carcass measurements, visual analyses of longissimus muscle color, color spectrophotometry (L*, a*, and b*), percentage drip loss, and ultimate pH were obtained from each pig at 24-h postmortem using the same procedures as Exp. 1.

A 10 g tissue sample was obtained from the longissimus muscle at the 11th rib to measure transmission value. The sample was thoroughly mixed with 20 mL of distilled water and stored at 3 ^{o}C for 24 h. It was then centrifuged at 500 × g for 20 min and the supernatant was filtered through #1 Whatman filter paper. The filtrate (1 mL) was mixed with citric acid-

phosphate buffer (5 mL), stored for 30 min at 24 ^{o}C, and percentage turbidity measured at 600 nm. High transmission values indicate less soluble protein and low quality muscle.

Experiment 3

One thousand one hundred four barrows (initially 44.0 kg) were allotted by weight in a randomized complete block to each of the 8 experimental treatments arranged in a 2 × 2 × 2 factorial. There were 23 pigs per pen and 6 replicates per treatment. The main effects included dietary L-carnitine (0 or 50 ppm), RAC (0 or 10 ppm), and added fat (0 or 6%).

Pigs were fed a corn-soybean meal diet (Table 1) with or without added L-carnitine and with or without added fat from 44 kg until slaughter (approximately 118 kg). Dietary RAC treatments (0 or 10 ppm) were fed for the last 4 wk of the experiment. The basal diet was formulated on a total lysine:calorie ratio basis with ratios of 3.16 g/Mcal ME from 44.0 to 61.2 kg, 2.70 g/Mcal ME from 61.2 to 92.1 kg, and 3.00 g/Mcal ME from 92.1 kg until the end of the experiment. The corresponding total lysine levels in the 0 and 6% added fat diets were 1.05 and 1.14% (17.6 and 18.5% CP); 0.90 and 0.97% (15.6 and 16.3% CP), and 1.00 and 1.08% (17.0 and 17.8% CP) total lysine for the three phases, respectively.

At the end of the experiment, 8 pigs were randomly selected from each pen and slaughtered at a commercial facility. Standard carcass measurements, visual analyses of longissimus muscle color, marbling, and firmness, longissimus muscle area, color spectrophotometry (L*, a*, and b*), percent drip loss, and ultimate pH were obtained from each pig at approximately 24-h postmortem.

Statistical Analyses

Data from all experiments were analyzed as a randomized complete block design with pen as the experimental unit for carcass characteristics and meat quality measurements. Pigs were blocked based on initial weight at the beginning of each experiment, and analysis of variance was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Linear and quadratic polynomial contrasts were performed to determine the effects of L-carnitine level (Exp. 1) and RAC level (Exp. 1 and 2). Hot carcass weight was used as a covariate in the statistical analysis of backfat, carcass length, longissimus muscle area, and percentage lean.

Results

Experiment 1

There were no RAC × L-carnitine interactions observed ($P > 0.14$) for carcass characteristics (Table 2). Dressing percentage tended to be greater ($P < 0.08$) for pigs fed RAC compared to control pigs. Shrink loss (1-(cold carcass wt/hot carcass wt)×100), average backfat, tenth rib fat depth, carcass length, longissimus muscle area, percentage lean were not affected ($P > 0.12$) by RAC or dietary L-carnitine.

A RAC × L-carnitine interaction was observed ($P < 0.02$) for visual color, L*, a*/b* ratio, and Hue angle (Table 3). Added L-carnitine did not improve visual color scores in control pigs, but L-carnitine improved visual color when 5 or 10 ppm of RAC was fed. Pigs fed increasing L-carnitine had lower L* values when fed with 5 or 10 ppm of RAC, resulting in a darker colored longissimus muscle measured at the 10^{th} rib. Adding L-carnitine to diets containing 5 or 10 ppm RAC, but not to control diets, decreased a*/b* and Hue angle values, resulting in more red and less orange color.

Measurements of b* decreased (quadratic, $P < 0.05$) with increasing L-carnitine, resulting in less yellow color of the longissimus muscle. The saturation index (vividness or intensity) measured on the longissimus muscle tended to decrease (quadratic, $P < 0.07$) with increasing levels of L-carnitine. Drip loss measured 48 h postmortem and longissimus temperature at 45 min postmortem decreased (linear, $P < 0.04$) with increasing L-carnitine. Ultimate (24-h) pH increased (linear, $P < 0.07$) with increasing dietary L-carnitine. Twenty-four h pH increased and then decreased (quadratic, $P < 0.06$) with increasing RAC and was highest for pigs fed 5 ppm.

Experiment 2

A RAC × L-carnitine interaction was observed ($P < 0.01$) for dressing percentage (Table 4). Dressing percentage was higher for pigs fed 25 ppm L-carnitine than pigs fed other L-carnitine levels and no RAC, but was lower for pigs fed 25 ppm L-carnitine than other L-carnitine levels and 10 ppm RAC.

Shrink loss, carcass length, and longissimus muscle area were not affected ($P > 0.37$) by feeding either RAC or L-carnitine. Tenth rib fat depth and average backfat were not affected ($P > 0.30$) by feeding RAC; however, there was a trend (linear, $P < 0.07$) for pigs fed increasing L-

carnitine to have lower 10th rib and average backfat. Feeding RAC to pigs increased ($P < 0.01$) percentage lean.

A RAC × L-carnitine interaction ($P < 0.04$) was observed for visual firmness, percentage drip loss, percentage transmission, and temperature measured 1.5 h postmortem (Table 5). Visual firmness scores decreased when pigs were fed increasing L-carnitine and no RAC but increased with increasing L-carnitine when RAC was in the diet. Percentage drip loss and transmission value decreased with increasing L-carnitine when fed with RAC. A low transmission value indicates less soluble protein and high quality muscle. Longissimus muscle temperature was lower for pigs fed increasing L-carnitine when RAC was fed.

Feeding L-carnitine to pigs did not affect ($P > 0.07$) other measured carcass characteristics. Visual color scores and a*/b* decreased ($P < 0.02$) and L* and hue angle increased ($P < 0.01$) when pigs were fed RAC, resulting in a lighter colored longissimus muscle. Pigs fed RAC also had higher temperature and lower pH measured 3 h postmortem ($P < 0.01$) and tended ($P < 0.06$) to have lower pH measured 6 h postmortem.

Experiment 3

An L-carnitine × RAC × fat interaction was observed ($P < 0.04$) for longissimus muscle area (Table 6). In general, adding RAC, L-carnitine, or fat to the diet increased longissimus muscle area; however, the responses were not entirely additive leading to the interaction.

An L-carnitine × RAC interaction ($P < 0.03$) was observed for fat thickness and percentage lean. Fat thickness decreased and lean percentage increased in pigs fed L-carnitine or RAC; however, neither of the responses were additive. Pigs fed added fat had greater ($P < 0.01$) fat thickness and lower percentage lean than pigs not fed added fat. A trend for an L-carnitine × RAC interaction ($P < 0.06$) also was observed for loin depth measured at the 10^{th} rib. Both added L-carnitine and RAC increased loin depth, but the response was not as great when L-carnitine and RAC were both added to the diet. Pigs fed added fat had decreased ($P < 0.01$) loin depth compared to pigs not fed added fat.

Carcass weight was greater ($P < 0.01$) for pigs fed 6% added fat and tended ($P < 0.07$) to be greater for pigs fed L-carnitine. A trend for an L-carnitine × RAC interaction ($P < 0.09$) was observed for 1st rib backfat. Pigs fed L-carnitine or RAC had decreased fat depth measured at the 1st rib, but when fed in combination, fat depth was not further decreased. Last lumbar

backfat was decreased ($P < 0.02$) in pigs fed either L-carnitine or RAC. Tenth rib and average backfat were decreased ($P < 0.03$) in pigs fed RAC compared to pigs not fed RAC. Pigs fed 6% added fat had greater ($P < 0.01$) 1st rib, last lumbar, and average backfat than pigs fed the diet without added fat.

An L-carnitine × fat interaction was observed ($P < 0.04$) for visual firmness scores. Visual firmness scores were improved in pigs fed L-carnitine and no added fat compared to pigs fed L-carnitine and 6% added fat.

Hunter a* values were greater ($P < 0.01$) for pigs fed RAC, resulting in more redness of the longissimus muscle. Hunter a* values were decreased ($P < 0.01$) for pigs fed added fat, resulting in less redness. Pigs fed 6% added fat also had increased ($P < 0.03$) b* values, which resulted in more yellowness of the longissimus muscle. Saturation index, or vividness, was greater ($P < 0.01$) for pigs fed diets containing 6% added fat and less ($P < 0.01$) for pigs fed RAC.

In contrast to Exp. 1 and 2, pigs fed RAC in this study had higher ($P < 0.04$) ultimate longissimus pH along with pigs fed the diet containing no added fat. In agreement with the pH data, pigs fed RAC had less ($P < 0.05$) drip loss using the filter paper method along with the pigs that were fed the diet with no added fat ($P < 0.02$). Pigs fed L-carnitine tended to have decreased ($P < 0.06$) percentage drip loss using the suspension method. The reduction in percentage drip loss with added L-carnitine agrees with the results of Exp. 1 and 2.

Discussion

The increase in longissimus muscle of pigs fed RAC is supported by reports of Weber et al. (1992) and Apple et al. (2008). In our experiments, longissimus muscle area was increased for pigs fed RAC in Exp. 3, but not different in Exp. 1 and 2, compared to pigs not fed RAC.

Inconsistencies in the effect of RAC on backfat have been reported. Stoller et al. (2003) observed that high-lean genetic pigs fed RAC had reduced 10^{th} rib backfat whereas 10^{th} rib backfat was not affected in Berkshire and Duroc pigs fed RAC. However, Schinckel et al. (2003a) observed a decrease in ultrasonic 10^{th} rib backfat and last-rib backfat for pigs fed RAC. Furthermore, the authors reported that pigs fed RAC in diets formulated to maximize lean growth had reduced lipid percentage of both the dissected fat and muscle tissue compared to pigs not fed RAC. The inconsistencies in decreased backfat are in agreement with our experiments in

which we observed a decrease in fat thickness, last-lumbar backfat, and last-rib backfat in Exp. 3 for pigs fed RAC; however, backfat was not affected by feeding RAC in Exp. 1 and 2.

Schinckel et al. (2003b) demonstrated that in pigs fed RAC, the requirement for dietary lysine for ADG is lower than the requirement for protein accretion, fat-free lean gain, and G:F. The authors theorized that pigs fed RAC require higher lysine concentrations than control pigs for two reasons: 1) ractopamine decreases feed intake and thus grams of lysine intake, and 2) the percentage of lysine in the protein is increased by RAC.

Other research feeding RAC to pigs has demonstrated that growth performance improvements occur with relatively short RAC supplementation durations (Williams et al., 1994; Schinckel et al., 2003b); however, improvements in lean tissue accretion, longissimus muscle area, and decreased backfat typically require a longer RAC supplementation duration (Schinckel et al., 2003a). In all of our experiments, RAC was fed for the last 4 wk before slaughter (109 and 118 kg in Exp. 1 and 2, and 3, respectively). It is currently not understood whether L-carnitine demonstrates a similar response.

Heo et al. (2000a) demonstrated that carnitine is essential for intermediary energy metabolism. A major function of carnitine is to transport long chain fatty acid groups across the mitochondrial membrane into the mitochondrial matrix for energy production (adenosine triphosphate) via β-oxidation and oxidative phosphorylation. In agreement, Owen et al. (2001a) observed that supplemental L-carnitine promoted fatty acid oxidation through accelerated β-oxidation, decreased flux of mitochondrial branched chain keto acid dehydrogenase activity, and increased flux through pyruvate carboxylase in pigs which suggests that pigs fed L-carnitine are more able to use fat for energy, divert carbon toward synthesis of amino acids, and spare branched-chain amino acids for protein synthesis.

Studies evaluating added L-carnitine with added dietary fat in pig diets (Owen et al., 1996; Heo, et al., 2000b, Apple et al., 2008) have yielded inconsistent results. Because of carnitine's known function of transporting fatty acids across the mitochondrial membrane, its affect may be different depending on the energy density of the diet. Adding L-carnitine to the diet could increase the amount of energy available for protein deposition and increase the pigs' response to RAC.

In young pigs (17.8 kg), carcass protein accretion increased and percentage fat in the carcass decreased when L-carnitine was added to the diet (Heo et al., 2000b). These results are

similar to those reported by Owen et al. (1996) who observed that pigs (initially, 6.0 kg) fed 1,000 ppm L-carnitine had less carcass lipid and daily lipid accretion on d 35 compared to pigs not fed L-carnitine; however, carcass moisture and CP percentages were not influenced by dietary L-carnitine.

In growing-finishing pigs (56.3 to 103 kg), Owen et al. (2001b) observed that pigs fed increasing L-carnitine (0, 25, 50, 75, 100, and 125 ppm) had decreased average and 10th-rib backfat, and increased percentage lean and daily CP accretion rate. However, in their study there was no effect of dietary treatment on longissimus muscle area or visual scores for longissimus color and firmness.

Owen et al. (2001a) observed a linear decrease in 10^{th} rib backfat thickness and increased percentage lean and muscle with increasing levels of L-carnitine (0, 50, or 125 ppm) in pigs from 56 to 120 kg. In their study, visual scores for carcass muscling, longissimus muscle marbling, and firmness were not affected by dietary treatment; however, increasing dietary L-carnitine increased visual color scores for longissimus muscle color (darker meat).

In an experiment conducted by Stoller et al. (2003) there was no effect of added RAC on visual color, firmness, or marbling. It is not surprising that visual observations were not affected in their experiment because RAC did not affect longissimus muscle pH or drip loss percentage.

In a study conducted by Smith et al. (1996), the authors observed no differences in backfat, longissimus muscle area, or percentage lean in pigs fed L-carnitine. However, these authors did observe an interaction between L-carnitine and chromium for visual longissimus color and firmness scores. Pigs fed either L-carnitine or chromium alone were not affected by treatment, but pigs fed both L-carnitine and chromium had darker and firmer visual longissimus muscle scores.

Trapp et al. (2002) observed an increase in hot carcass wt for gilts fed either 50 ppm L-carnitine and 5 or 10 ppm RAC compared to pigs fed the control diet or diets with 50 ppm L-carnitine. Pigs fed RAC had increased loin depth, decreased backfat, and increased percentage lean. In their study, marbling and firmness scores did not differ among treatments; however, pigs fed 50 ppm L-carnitine and 5 ppm RAC had increased visual color scores resulting in a darker colored meat. In addition, pigs fed 50 ppm L-carnitine and 5 ppm RAC had increased longisimuss muscle area compared to pigs fed the control diet or diets containing 50 ppm L-carnitine.

Feeding L-carnitine improves meat quality traits when fed in combination with RAC. The improvements may be the result of L-carnitine's affect on the pigs' metabolism either pre- or post-mortem. Supplemental L-carnitine has been reported to influence the enzymes involved in lactic acid production. Pyruvate carboxylase increases and lactate dehydrogenase decreases in pigs fed added L-carnitine (Owen et al., 2001a). Xi et al. (2008) fed sows L-carnitine and observed increased fetal pyruvate carboxylase complex in the heart and liver. An increase in pyruvate carboxylase may direct pyruvate away from lactate, thus reducing substrate available for lactic acid synthesis postmortem. Furthermore, a decrease in lactate dehyrogenase may delay the onset of postmortem glycolysis. A reduction in post-mortem lactic acid production would increase pH, and therefore improve meat quality by increasing water holding capacity and decreasing percentage drip loss. Baumgartner and Blum (1998) reported that pigs fed 25 ppm L-carnitine had decreased pH measured 30 m post-mortem. Subsequently, meat color was darker.

In summary, these results suggest that L-carnitine improves meat quality in pigs fed RAC. Further research needs to be conducted to better understand the effects and metabolic action of L-carnitine on antimortem lactate levels and postmortem glycolysis. However, if further studies confirm pork quality benefits, such as decreased drip loss, increased pH, and improved meat color, or decreased serum lactate levels, the potential exists for dietary L-carnitine to be used in conjunction with RAC in the late finishing diet.

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Table 3.1 Basal diet composition (as-fed basis)[a]

	Exp. 1[b]		Exp. 2[c]	Exp. 3[d]						Exp.4[d]
Initial BW, kg:	33	74	87	44		61		92		103
Ingredient, %				No Fat	Fat	No Fat	Fat	No Fat	Fat	
Corn	68.41	74.50	74.50	73.00	63.30	78.60	69.35	75.10	65.55	75.10
Soybean meal, 46.5 % CP	26.63	22.80	22.80	24.60	28.25	19.15	22.35	22.75	26.25	22.75
Choice white grease	-	-	-	-	6.00	-	6.00	-	6.00	-
Soybean oil	2.00	-	-	-	-	-	-	-	-	-
Monocalcium phosphate, 21% P	1.05	0.90	0.90	0.85	0.94	0.73	0.80	0.64	0.70	0.64
Limestone	1.00	0.90	0.90	0.88	0.84	0.85	0.83	0.84	0.81	0.84
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[e]	0.15	0.15	0.15	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Trace mineral premix[f]	0.15	0.15	0.15	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine·HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Medication[g]	0.05	-	-	-	-	-	-	-	-	-
DL-Methionine	0.01	-	-	-	-	-	-	-	-	-
Cornstarch	0.05	0.10	0.10	-	-	-	-	-	-	-
Calculated composition										
CP (N × 6.25), %	18.20	16.90	16.90	17.60	18.50	15.60	16.30	17.00	17.80	17.00
Lysine, %	1.10	1.00	1.00	1.05	1.14	0.90	0.97	1.00	1.08	1.00
Lysine:calorie ratio, g/mcal	3.24	3.01	3.01	3.16	3.16	2.70	2.70	3.00	3.00	3.00
ME, kcal/kg	3,399	3,318	3,318	3,327	3,596	3,336	3,605	3,338	3,607	3,338
Ca, %	0.69	0.61	0.61	0.60	0.61	0.55	0.56	0.54	0.55	0.54
P, %	0.60	0.55	0.55	0.55	0.57	0.50	0.52	0.50	0.51	0.50

[a] Diets were formulated to meet or exceed NRC (1998) requirements.

[b] L-carnitine replaced cornstarch to provide either 0, 25, or 50 ppm L-carnitine. RAC replaced cornstarch to provide either 0, 5, or 10 ppm ractopamine·HCl.

[c] L-carnitine replaced cornstarch to provide starch either 0, 25, or 50 ppm L-carnitine. RAC replaced cornstarch to provide 0 or 10 ppm ractopamine·HCl.

[d] L-carnitine replaced corn to provide either 0, or 50 ppm L-carnitine and RAC replaced corn to provide 0 or 10 ppm ractopamine·HCl.

[e] In Exp. 1 and 2, vitamin premix provided (per kilogram of complete diet): vitamin A, 6,614 IU; vitamin D_3, 992 IU; vitamin E, 26.5 IU; menadione (menadione dimethylpyrimidinol bisulphite), 2.65; vitamin B_{12}, 0.03 mg; riboflavin, 5.95 mg; pantothenic acid, 19.8 mg; and niacin, 33.1 mg. In Exp. 3 and 4, vitamin premix provided (per kilogram of complete diet): vitamin A, 7,937 USP; vitamin D_3, 1,190 USP; vitamin E, 31.75 IU, vitamin B_{12}, 0.03 mg; riboflavin, 7.14 mg; pantothenic acid, 23.81 mg; niacin, 39.68 mg.

[f] In Exp. 1 and 2,, trace mineral premix provided (per kilogram of complete diet): Mn (from manganese oxide), 39.7 mg; Fe (from ferrous sulfate), 165.3 mg; Zn (from zinc oxide) 165.3 mg; Cu (from copper sulfate), 16.5 mg; I (from calcium iodate) 0.3 mg; and Se (from sodium selenite), 0.3 mg. In Exp. 3 and 4, trace mineral premix provided (per kilogram of complete diet): Mn (from manganese oxide), 35.7 mg; Fe (from ferrous sulfate), 148.8 mg; Zn (from zinc oxide), 148.8 mg; Cu (from copper sulfate), 14.9 mg ; I (from calcium iodate) 0.3 mg; and Se (from sodium selenite) 0.3 mg.

[g] Provided 44 mg tyloslin per kg diet.

Table 3.2 Carcass characteristics of finishing pigs fed L-carnitine and Ractopamine HCl (Exp. 1)[a,b]

	Ractopamine HCl, ppm										Probability ($P <$)						
	0			5			10										
	L-carnitine, ppm										Ractopamine HCl ×	Ractopamine		Ractopamine HCl		L-carnitine	
Item	0	25	50	0	25	50	0	25	50	SED	L-carnitine	HCl	L-carnitine	Linear	Quad[c]	Linear	Quad[c]
Dressing, %	72.99	73.39	73.40	74.19	74.26	73.68	75.18	73.40	73.63	0.57	0.14	0.08	0.40	0.06	0.25	0.21	0.64
Shrink loss[d], %	2.15	2.12	2.13	2.15	2.64	2.08	1.32	2.01	1.96	0.40	0.69	0.13	0.37	0.53	0.05	0.45	0.24
Backfat, cm																	
First rib	3.95	3.63	3.82	3.69	3.67	3.73	3.62	3.66	3.50	0.25	0.87	0.51	0.82	0.55	0.32	0.65	0.67
Tenth rib	1.69	1.45	1.52	1.44	1.40	1.35	1.47	1.45	1.18	0.14	0.64	0.13	0.19	0.10	0.22	0.07	0.96
Last rib	2.55	2.45	2.39	2.31	2.49	2.42	2.63	2.45	2.27	0.16	0.60	0.86	0.46	0.61	0.85	0.23	0.70
Last lumbar	1.74	1.63	1.71	1.53	1.41	1.69	1.53	1.45	1.53	0.16	0.94	0.20	0.43	0.16	0.27	0.77	0.21
Average	2.75	2.57	2.64	2.51	2.52	2.62	2.60	2.53	2.43	0.17	0.86	0.48	0.74	0.34	0.45	0.55	0.63
Carcass length, cm	80.04	80.16	79.60	80.65	79.54	79.84	79.10	79.51	79.92	0.70	0.53	0.56	0.93	0.85	0.29	0.80	0.77
Loin eye area, cm^2	43.94	45.83	42.60	44.44	44.75	46.05	46.90	47.09	46.94	2.47	0.88	0.23	0.85	0.62	0.10	0.99	0.57
Lean, %	56.17	58.04	56.56	57.47	57.96	58.50	58.30	59.72	59.72	1.31	0.78	0.12	0.52	0.26	0.09	0.31	0.64

[a] Hot carcass weight was used as a covariate in the statistical analysis except for dressing (%) and shrink loss (%).

[b] Values are means of seven or five replications (pig closest to 118 kg in each pen).

[c] Quad (quadratic).

[d] Shrink loss was calculated as 1-(cold carcass wt/hot carcass wt) × 100.

Table 3.3 Carcass quality measures of finishing pigs fed L-carnitine and Ractopamine HCl (Exp 1)[a]

	Ractopamine HCl, ppm										Probability ($P <$)						
	0			5			10										
	L-carnitine, ppm										Ractopamine HCl ×	Ractopamine		Ractopamine HCl		L-carnitine	
Item	0	25	50	0	25	50	0	25	50	SED	L-carnitine	HCl	L-carnitine	Linear	Quad[c]	Linear	Quad[c]
Visual color[c]	3.35	2.78	3.14	2.57	3.28	3.49	2.57	3.00	2.85	0.25	0.02	0.15	0.18	0.99	0.08	0.11	0.82
Firmness[c]	1.93	1.65	1.93	1.79	1.93	2.05	1.79	2.15	1.79	0.24	0.43	0.88	0.88	0.66	0.81	0.66	0.81
Marbling[c]	2.00	1.71	1.85	1.35	2.07	1.82	1.64	2.00	1.71	0.21	0.08	0.76	0.22	0.46	0.91	0.42	0.13
L*[d]	55.37	58.01	56.80	60.78	56.39	55.06	61.53	58.46	57.88	1.25	0.01	0.01	0.02	0.41	0.01	0.01	0.68
a*[d]	7.61	6.17	6.45	5.78	6.22	7.00	6.30	6.71	6.51	0.53	0.08	0.49	0.81	0.23	0.99	0.94	0.52
b*[d]	15.25	14.61	15.10	15.69	14.09	14.19	15.90	14.98	15.04	0.53	0.67	0.25	0.01	0.42	0.14	0.03	0.05
a*/b*[d]	0.50	0.42	0.43	0.37	0.44	0.50	0.39	0.45	0.43	0.03	0.01	0.52	0.33	0.42	0.49	0.27	0.90
Hue angle	63.60	67.38	67.05	69.95	66.31	63.71	68.65	65.91	66.69	1.44	0.01	0.55	0.31	0.44	0.52	0.25	0.84
Saturation index[d]	17.06	15.89	16.44	16.76	15.42	15.84	17.12	16.43	16.41	0.64	0.97	0.32	0.04	0.30	0.26	0.09	0.07
Drip loss, %	2.68	2.80	2.07	3.13	1.48	1.49	3.68	2.29	2.94	0.66	0.47	0.16	0.06	0.33	0.09	0.04	0.22
Temperature, °C	34.72	34.83	32.98	34.39	34.38	33.80	35.72	34.15	33.76	0.83	0.60	0.74	0.04	0.97	0.44	0.01	0.62
pH																	
45 m postmortem	6.22	6.55	6.46	6.41	6.44	6.34	6.33	6.23	6.39	0.10	0.10	0.38	0.39	0.99	0.17	0.24	0.49
24 h postmortem	5.75	5.79	5.76	5.79	5.86	5.86	5.71	5.79	5.78	0.04	0.91	0.01	0.04	0.01	0.06	0.07	0.08

[a] Values are means of seven or five replications (pig closest to 240 lb in each pen).

[b] Scoring system of 1 to 5: 2 = grayish pink, traces to slight, or soft and watery; 3 = reddish pink, small to modest, or slightly firm and moist; and 4 = purplish red, moderate to slightly abundant, or firm and moderately dry for color, firmness, and marbling, respectively.

[c] Quad (quadratic)

[d] Means were derived from two sample readings per loin. Measures of dark to light (L*), redness (a*), yellowness (b*), red to orange (hue angle), or vividness or intensity (saturation index).

Table 3.4 Carcass characteristics of finishing pigs fed L-carnitine and Ractopamine HC1 (Exp. 2)[a,b]

	Ractopamine HCl, ppm											
	0			10				Probability ($P <$)				
	L-carnitine, ppm							Ractopamine HCl ×			L-carnitine	
Item	0	25	50	0	25	50	SED	L-carnitine	Ractopamine HCl	L-carnitine	Linear	Quadratic
Dressing, %	72.30	74.48	72.71	74.90	73.56	74.25	0.39	0.01	0.35	0.01	0.79	0.23
Shrink loss[c], %	2.27	1.72	1.73	1.76	1.79	1.74	0.24	0.41	0.46	0.42	0.24	0.56
Backfat, cm												
First rib	3.57	3.58	3.16	3.62	3.77	3.42	0.18	0.83	0.25	0.08	0.09	0.14
Tenth rib	1.73	1.65	1.60	1.69	1.60	1.43	0.11	0.77	0.30	0.18	0.06	0.86
Last rib	2.06	2.10	2.02	2.27	1.98	2.00	0.14	0.47	0.84	0.46	0.24	0.70
Last lumbar	1.84	1.66	1.74	1.80	1.66	1.49	0.13	0.85	0.35	0.26	0.11	0.63
Average	2.49	2.44	2.30	2.56	2.46	2.30	0.12	0.96	0.76	0.19	0.07	0.73
Carcass length, cm	82.94	83.38	82.68	82.88	83.00	82.94	0.66	0.89	0.90	0.83	0.88	0.55
Loin eye area, cm^2	42.56	45.76	43.40	50.40	46.50	50.98	1.47	0.94	0.76	0.37	0.65	0.60
Lean, %	54.85	55.50	55.80	56.71	56.19	58.09	0.71	0.50	0.01	0.19	0.10	0.40

[a] Hot carcass weight was used as a covariate in the statistical analysis except for dressing (%) and shrink loss (%).

[b] Values are means of ten replications (one pig selected randomly from each pen).

[c] Shrink loss was calculated as 1-(cold carcass wt/hot carcass wt) × 100.

Table 3.5 Carcass characteristics of finishing pigs fed L-carnitine and Ractopamine HC1 (Exp. 2)[a]

	Ractopamine HCl, ppm							Probability ($P <$)				
	0			10								
	L-carnitine, ppm							Ractopamine HCl ×	Ractopamine		L-carnitine	
Item	0	25	50	0	25	50	SED	L-carnitine	HCl	L-carnitine	Linear	Quadratic
Visual color[b]	3.20	3.10	2.90	2.75	2.75	2.80	0.16	0.52	0.02	0.72	0.93	0.43
Firmness[b]	2.59	2.44	2.34	1.99	2.59	2.34	0.15	0.04	0.25	0.33	0.75	0.15
Marbling[b]	1.65	1.75	1.55	1.85	1.75	1.60	0.18	0.85	0.57	0.53	0.33	0.57
L*[c]	57.18	57.23	58.00	59.72	59.63	58.44	0.83	0.37	0.01	0.95	0.78	0.89
a*[c]	7.54	7.58	7.93	7.94	6.73	6.61	0.38	0.07	0.07	0.29	0.24	0.30
b*[c]	15.81	15.86	16.27	16.97	15.75	15.29	0.47	0.08	0.95	0.37	0.22	0.51
a*/b*[c]	0.48	0.48	0.49	0.46	0.43	0.43	0.02	0.40	0.01	0.44	0.46	0.30
Hue angle[c]	64.49	64.61	64.07	65.14	66.99	66.70	0.78	0.39	0.01	0.45	0.47	0.30
Saturation index[c]	17.52	17.59	18.11	18.74	17.13	16.67	0.56	0.06	0.64	0.321	0.20	0.42
Drip loss, %	2.04	3.07	2.73	4.85	2.47	2.82	0.64	0.02	0.48	0.15	0.32	0.56
Transmission, %	50.40	53.09	60.00	66.69	49.85	58.27	3.52	0.01	0.19	0.06	0.87	0.02
Temperature, °C												
5 min postmortem	38.59	39.79	39.17	39.60	39.74	39.68	0.56	0.60	0.24	0.40	0.51	0.25
15 min postmortem	40.20	39.92	39.56	40.42	40.18	40.06	0.31	0.88	0.16	0.23	0.09	0.97
45 min postmortem	37.72	39.03	38.73	39.43	38.35	38.76	0.69	0.18	0.16	0.23	0.73	0.93
1.5 h postmortem	32.91	32.87	33.17	35.99	33.51	32.65	0.72	0.04	0.06	0.07	0.05	0.46
3 h postmortem	21.38	22.12	20.89	24.38	22.37	22.76	0.63	0.10	0.01	0.26	0.11	0.85
6 h postmortem	10.74	11.28	11.22	12.04	11.09	10.97	0.48	0.20	0.47	0.82	0.55	0.89
Blood												
Glucose, mg/dL	109.73	107.07	108.44	109.21	103.82	108.89	3.74	0.88	0.71	0.51	0.83	0.26
Lactate, mmol/L	12.46	11.78	10.41	11.71	9.93	10.36	1.51	0.84	0.47	0.50	0.26	0.77
pH	7.14	7.13	7.21	7.16	7.16	7.21	0.05	0.94	0.76	0.37	0.23	0.47
Longissimus pH												
5 min postmortem	6.93	6.84	6.82	6.79	6.80	6.94	0.07	0.17	0.76	0.66	0.74	0.40
15 min postmortem	6.55	6.60	6.58	6.59	6.47	6.49	0.07	0.48	0.35	0.84	0.62	0.76
45 min postmortem	6.16	6.16	6.02	6.14	6.21	6.13	0.10	0.82	0.57	0.54	0.44	0.43
1.5 h postmortem	5.95	5.91	5.97	5.89	5.95	5.92	0.10	0.87	0.74	0.96	0.77	0.94
3 h postmortem	5.77	5.76	5.88	5.59	5.67	5.69	0.08	0.77	0.01	0.33	0.15	0.73
6 h postmortem	5.76	5.70	5.70	5.61	5.66	5.68	0.04	0.23	0.06	0.98	0.90	0.89
24 h postmortem	5.64	5.61	5.60	5.58	5.64	5.59	0.02	0.19	0.57	0.46	0.60	0.26

[a] Values are means of ten replications (one pig selected randomly from each pen).

[b] Scoring system of 1 to 5: 2 = grayish pink, traces to slight, or soft and watery; 3 = reddish pink, small to modest, or slightly firm and moist; and 4 = purplish red, moderate to slightly abundant, or firm and moderately dry for color, firmness, and marbling, respectively.

[c] Means were derived from two sample readings per loin. Measures of dark to light (L*), redness (a*), yellowness (b*), red to orange (hue angle), or vividness or intensity (saturation index).

Table 3.6 Interactive effects of L-carnitine, Ractopamine HC1, and fat on carcass characteristics and meat quality of finishing pigs (Exp. 3)[a]

	Fat, %															
	0				6											
	L-carnitine, ppm															
	0		50		0		50			Probability ($P <$)						
	Ractopamine HCl, ppm									L-carnitine × Ractopamine HCl×Fat	L-carnitine × Ractopamine HCl	L-carnitine × Fat	Ractopamine HCl × Fat	L-carnitine	Ractopamine HCl	Fat
Item	0	10	0	10	0	10	0	10	SED							
Carcass wt, lb	197.96	201.44	200.27	203.44	203.48	207.10	210.06	209.64	2.62	0.64	0.53	0.48	0.67	0.07	0.19	0.01
Fat thickness, mm[b]	16.76	13.92	16.29	14.72	18.83	16.09	16.77	16.25	0.56	0.54	0.03	0.16	0.47	0.32	0.01	0.01
Loin depth, mm[b]	59.28	62.80	60.89	61.52	57.93	61.11	59.16	60.65	0.81	0.68	0.06	0.94	0.91	0.54	0.01	0.01
Lean,%[c]	56.15	59.19	56.82	58.29	54.12	57.03	56.13	56.82	0.59	0.67	0.02	0.23	0.56	0.33	0.01	0.01
Loin eye area, cm^2	47.34	48.59	48.47	50.25	47.24	51.33	52.09	50.48	1.10	0.04	0.09	0.69	0.85	0.03	0.07	0.03
Backfat, cm																
First rib	3.58	3.49	3.57	3.49	3.91	3.62	3.57	3.66	0.08	0.13	0.09	0.22	0.89	0.19	0.11	0.01
Tenth rib	1.67	1.58	1.69	1.63	1.74	1.65	1.64	1.64	0.04	0.53	0.29	0.12	0.53	0.70	0.03	0.47
Last rib	2.68	2.53	2.70	2.67	2.84	2.76	2.81	2.82	0.07	0.89	0.30	0.49	0.57	0.36	0.18	0.01
Last lumbar	1.55	1.41	1.51	1.39	1.70	1.60	1.53	1.46	0.06	0.93	0.74	0.11	0.54	0.02	0.01	0.01
Average backfat	2.61	2.48	2.60	2.52	2.81	2.66	2.64	2.63	0.05	0.51	0.17	0.09	0.72	0.19	0.01	0.01
Visual color[c]	3.39	3.18	3.48	3.38	3.38	3.48	3.45	3.26	0.09	0.14	0.62	0.09	0.44	0.43	0.26	0.69
Firmness[c]	2.50	2.96	2.86	2.98	2.70	2.76	2.48	2.64	0.13	0.26	0.48	0.04	0.28	0.83	0.03	0.05
Marbling[c]	2.44	2.51	2.45	2.41	2.46	2.43	2.18	2.50	0.15	0.27	0.60	0.78	0.53	0.46	0.47	0.56
L*[d]	45.44	45.73	45.28	46.14	45.29	45.81	46.31	46.45	0.43	0.42	0.78	0.27	0.64	0.10	0.10	0.32
a*[d]	6.07	5.52	6.18	5.48	6.53	5.96	6.41	5.72	0.16	0.96	0.63	0.27	0.88	0.62	0.01	0.01
b*[d]	0.97	0.95	0.92	0.83	1.05	1.12	1.29	1.28	0.16	0.87	0.94	0.29	0.77	0.48	0.91	0.03
a:b	4.64	1.86	-1.43	9.59	7.38	-15.14	3.86	2.52	6.96	0.70	0.06	0.51	0.09	0.39	0.41	0.40
Hue angle	8.88	9.20	7.52	7.03	8.95	9.80	10.64	11.23	1.39	0.99	0.99	0.14	0.78	0.91	0.58	0.06
Saturation index	6.24	5.69	6.34	5.65	6.67	6.16	6.60	5.99	0.18	0.95	0.74	0.46	0.91	0.81	0.01	0.01
Longissimus pH	5.59	5.61	5.62	5.62	5.57	5.60	5.55	5.61	0.02	0.39	0.96	0.34	0.13	0.54	0.04	0.04
Drip loss, %																
Filter paper	4.51	4.17	4.71	4.75	5.21	4.91	5.64	4.45	0.32	0.13	0.63	0.34	0.16	0.36	0.05	0.02
Suspension	6.92	6.52	5.81	6.07	7.29	6.65	6.98	6.22	0.41	0.48	0.56	0.52	0.27	0.06	0.22	0.12

[a]Values are means of six replications (pens) and eight pigs per pen.
[b]Measurements were determined with UFOM and collected 7 cm off the midline at the 10th rib, lean percentage was calculated with these values.
[c]Scoring system of 1 to 5: 2 = grayish pink, traces to slight, or soft and watery; 3 = reddish pink, small to modest, or slightly firm and moist; and 4 = purplish red, moderate to slightly abundant, or firm and moderately dry for color, firmness, and marbling, respectively.
[d]Measures of dark to light (L*), redness (a*), yellowness (b*).

CHAPTER 4 - Effect of Dietary L-Carnitine and Ractopamine·HCl on the Metabolic Response to Handling in Grow-Finish Pigs

Abstract

Two experiments (384 pigs; PIC C22 × L326) were conducted to determine the interactive effect of dietary L-carnitine and ractopamine·HCl (RAC) on the metabolic response to handling. Experiments were arranged as split plots with handling as the main plot and diet as subplots (4 pens per treatment). Dietary L-carnitine (0 or 50 ppm) was fed from 38.5 kg to the end of the trials (118 kg) and RAC (0 or 20 ppm) was fed for the last 4 wk of each trial. At the end of each trial, 4 pigs per pen were assigned to 1 of 2 handling treatments. Gentle-handled pigs were moved at a moderate pace 3 times through a 50 m course and up and down a 15° loading ramp. Non-gentle handled pigs were moved at a faster pace, up and down a 30° ramp, and were shocked 3 times by an electrical prod. Blood was collected immediately before and after handling in Exp. 1 and immediately after and 1 h after handling in Exp. 2. Feeding RAC increased ($P < 0.01$) ADG and G:F; however, there was no ($P > 0.10$) effect of L-carnitine on growth performance in either trial. In Exp. 1 and 2, non-gentle handling increased ($P < 0.01$) plasma lactate dehydrogenase (LDH), lactate, cortisol, and rectal temperature, and decreased blood pH. In Exp. 1, a RAC × handling interaction ($P < 0.06$) was observed for the difference in pre- and post-handling pH and temperature. Non-gentle handled pigs fed RAC had decreased pH and increased temperature and tended ($P < 0.09$) to have higher lactate than pigs gentle handled and fed RAC. Pigs fed RAC had increased ($P < 0.01$) LDH compared to pigs not fed RAC. Pigs fed L-carnitine had increased ($P < 0.03$) lactate compared to pigs not fed L-carnitine. In Exp. 2, pigs fed RAC had lower ($P < 0.02$) pH immediately after handling but pH returned to control levels ($P > 0.96$) by 1 h post-handling. Lactate, LDH, cortisol, and temperature changes from immediately post-handling to 1 h post-handling were not different for pigs fed L-carnitine or RAC suggesting that L-carnitine did not decrease recovery time of pigs subjected to non-gentle handling. These results suggest that pigs fed RAC are more susceptible to stress when handled aggressively compared to pigs not fed RAC. Dietary L-carnitine did not alleviate the effects of stress when fed in combination with RAC.

Key words: L-carnitine, Pigs, Ractopamine HCl

Introduction

The increased incidence of downer pigs and metabolic acidosis has been well recognized as an industry problem and has resulted in substantial economic loss. Approximately 0.5 to 0.1% of pigs are characterized as downer pigs in commercial pork processing plants (Carr et al., 2005). The economic consequences of pigs that die or become non-ambulatory during transportation or at the packing plant cost the U. S. swine industry about $100 million annually (Ellis et al., 2003). A downer pig has been categorized as a pig that becomes fatigued, refuses to get up and walk, or can't keep up with its contemporaries while loading, unloading, or moving through the packing plant (Grandin, 1998). The prevalence of downer pigs has been attributed to several factors including animal handling, genetics, and muscling. The occurrence of downer pigs may be amplified due to the industry trend of producing a more heavily muscled, lean pig (Grandin, 1998).

Non-gentle handling of pigs results in increased levels of serum lactate, decreased pH, and increased incidence of downer pigs (Anderson et al., 2002). Swine can be stressed resulting from numerous factors such as genetics, carcass muscling, health status, structural soundness, BW, nutrition, handling, facility design, and conditions during transport to the plant (Ritter et al., 2006). Previous research (James et al., 2009b) has suggested that supplemental L-carnitine may improve pork quality in pigs fed ractopamine HCl (RAC). The improvements in meat quality may be the result of L-carnitine's affects on either pre- or post-mortem metabolism. Dietary L-carnitine has been shown to increase pyruvate carboxylase and decrease lactate dehydrogenase in pigs (Owen et al., 2001). An increase in pyruvate carboxylase may direct pyruvate away from lactate, thus reducing substrate for lactic acid synthesis. Furthermore, a decrease in lactate dehydrogenase may delay the onset of glycolysis. In theory, this would result in an increase in pH.

Bertol et al. (2005) reported that pigs fed L-carnitine had reduced changes in blood pH and SO_2 concentrations when subjected to vigorous handling procedures and electrical prod stimulation. Because of the known influence of L-carnitine on enzymes involved in lactic acid production, L-carnitine may be able to reduce the negative effects of stress from handling and transportation in commercial swine production. The objective of our experiment was to

determine the interactive effect of dietary L-carnitine and RAC on the metabolic response to handling.

Materials and Methods

General

Procedures used in these experiments were approved by the Kansas State University Animal Care and Use Committee (Protocol No. 2156) and were conducted at the Kansas State University Swine Teaching Research Unit. Pigs were housed in a modified open-front building with 50% solid concrete and 50% concrete slat flooring. Each 1.8-m × 4.9-m pen had a 2-hole dry self-feeder and a nipple waterer to allow ad libitum access to feed and water. A total of 384 barrows and gilts (C22 × L326, PIC, USA, Hendersonville, TN) were used in 2 experiments. All pigs were used for the growth performance criteria and a sub-sample of 128 pigs was used for the handling and stress data. In each experiment, 192 pigs were blocked by weight and ancestry (initially 36 kg, BW) in a split plot design with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). There were 12 pigs per pen and 16 pens (4 replications) per experiment. The 4 dietary treatments were arranged as a 2 × 2 factorial. Pigs were fed a corn-soybean meal diet (Table 1) with or without added L-carnitine (0 or 50 ppm) from 36 kg until the end of each experiment (118 kg). The basal diet was formulated to contain 1.20% total lysine from 36 to 54 kg (Phase I), and 1.00% total lysine from 54 to 86 kg and 86 to 118 kg (Phase II and III, respectively). Dietary RAC treatments (0 or 20 ppm) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg). In these experiments, we fed pigs 20 ppm RAC to demonstrate the maximum response to added RAC; however, this is no longer an FDA approved level. All diets were formulated to meet or exceed nutrient requirement estimates (NRC, 1998).

Growth Performance

Weights were obtained on all pigs and feed added and feeder weights were recorded every 14 d during the experiment until the last 4 wk, at which time measurements were recorded at the beginning (86 kg) and the end (118 kg) of the 4 wk-period to calculate ADG, ADFI, and G:F. Pigs were only weighed at the beginning and the end of the last 4 wk (RAC supplementation period) so that they did not become accustom to the routine of being handled.

Stress Model

The 2 handling treatments (gentle and non-gentle) were imposed at the end of the experiment (118 kg). There were 8 pigs from each diet (4 blocks and 2 pigs per pen) used for each handling treatment. One pig per pen in a block (1 pig from each dietary treatment) was subjected to the respective handling treatment at the same time (groups of 4 pigs). Two pigs from each pen were subjected to the gentle handling treatment and 2 pigs from each pen were subjected to the non-gentle handling treatment. Pigs were selected randomly from each pen. The 2 handling treatments were conducted consecutively to avoid circadian and ambient temperature bias. The handling portion of the study was conducted in a different room in the barn so that other pigs in pens did not become excited as the handling treatments were being conducted.

In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15 ^{o} loading ramp, using a sorting board at a moderate pace (Figure 1). At the top of the loading ramp, pigs were moved onto a hydraulic cart, turned around, and moved back down the loading ramp. The 50 m course consisted of moving pigs back and forth (3 laps for a total of 150 m) in the alleyway of the finishing barn.

In the non-gentle handling treatment, pigs were moved aggressively at a quicker pace through the course, including up and down a 30 ^{o} loading ramp. Panels divided the alleyway and narrowed, resulting in crowding, at one end to simulate a single chute to model commercial loading and slaughter facilities. Pigs were subjected to 3 (one-second) stimulations, by an electrical prod (The Green One HS200, Hot-Shot, Savage, MN), per time around the course. Using an electric prod provided short-term discomfort so that physiological and metabolic differences due to dietary treatment could be determined. The use of an electric prod provided the same level of stimulation to all pigs in that category. This served as a model for the stress that pigs incur as they are loaded and transported to and in slaughter facilities.

Rectal temperature was recorded and blood collected immediately before and after handling in Exp. 1 and was conducted immediately after and 1 h after handling in Exp. 2 because there were no differences observed prior to handling in Exp. 1. The blood was collected via anterior vena cava by a veterinarian so that samples could be obtained quickly to prevent additional stress. Pigs were restrained for blood collection using a snout snare and quickly released after blood collection. Pigs were restrained for less than approximately 30 s. Blood

samples were immediately placed on ice and transported to the Kansas State University College of Veterinary Medicine to be analyzed for serum lactate dehydrogenase (LDH), lactate, pH, glucose, urea N, PCO_2, PO_2, SO_2, HCO_3, Na^+, K^+, Cl^-, Ca^{++}, Mg^{++}, and cortisol using an autoanalyzer. The time elapsed from blood collection to arrival at the laboratory was approximately 15 min. In Exp. 1, heart rate was measured between periods of blood collection during the handling treatments by fitting the pigs with a Polar Vantage NV heart rate monitor (Polar Electro Oy, Kempele, Finland) to record and store successive interbeat intervals as described by Marchant et al. (1995). There were unequal observations across treatments because some of the connections between the heart rate monitors and the pigs became unstable.

Statistical Analyses

Data were analyzed as a split plot design with handling (gentle or non-gentle) as the whole plot and diet (L-carnitine, 0 or 50 ppm; and RAC, 0 or 20 ppm) as the subplot. In each experiment, there were 4 observations per treatment diet (pens) for growth performance. A sub-sampling of individual pigs (4 pigs per pen; 2 for gentle and 2 for non-gentle) were used for metabolic and physiological response data. Analysis of variance was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC).

Results

Combined Growth Performance

The growth performance data from Exp. 1 and 2 were combined (Table 2). There was no affect ($P > 0.40$) of feeding pigs L-carnitine on ADG, ADFI, or G:F from 36 to 86 kg (Pre-RAC). From d 0 to 28 of the RAC supplementation period there were no RAC × L-carnitine interactions ($P > 0.28$) or main effects of L-carnitine ($P > 0.58$) for any of the growth performance criteria. Pigs fed RAC had increased ($P < 0.01$) ADG and G:F. For the overall finishing period (36 to 118 kg), there were no RAC × L-carnitine interactions ($P > 0.53$) observed for ADG, ADFI, or G:F or main effects of L-carnitine. Pigs fed RAC had greater ($P < 0.01$) ADG and G:F compared to pigs not fed RAC.

Stress Model

Experiment 1. There were no pre-handling RAC × L-carnitine interactions ($P > 0.20$) on any of the pre-handling metabolite measurements (Table 3). There were no RAC × L-carnitine × handling interactions ($P > 0.14$) or RAC × L-carnitine interactions ($P > 0.15$) immediately post-handling or for the difference between pre-handling and post-handling for any of the criteria measured.

Pigs that were subjected to the non-gentle handling treatment or fed RAC had increased ($P < 0.01$) LDH concentration post-handling and had a greater ($P < 0.01$) difference (LDH increase) between pre-handling and post-handling compared to pigs that were handled gentle or not fed RAC. Adding L-carnitine to the diet had no effect ($P < 0.41$) on LDH concentrations.

Pigs fed RAC had increased ($P < 0.01$) pre-handling lactate concentration compared to pigs not fed RAC. Lactate concentration was highest post-handling for pigs that were non-gentle handled and fed RAC. This resulted in a RAC × handling interaction ($P < 0.09$) trend for the difference between pre-handling and post-handling lactate concentration. Although pigs fed RAC had higher ($P < 0.01$) pre-handling lactate concentration compared to pigs not fed RAC, lactate increased even more post-handling for pigs that were non-gentle handled. The difference in pre- and post-handling lactate concentration was greater ($P < 0.03$) in pigs fed L-carnitine compared with others.

Pigs fed RAC had slightly lower ($P < 0.04$) pre-handling pH compared to pigs not fed RAC. A RAC × handling interaction ($P < 0.01$) was observed for pH post-handling. Ractopamine HCl had no effect on pH in pigs that were handled gently; however, feeding RAC reduced pH in pigs that were not handled gently. This resulted in a RAC × handling interaction ($P < 0.05$) for the difference in pH between pre-handling and post-handling. The pH of pigs fed RAC was initially lower than pigs not fed RAC and pH decreased more for pigs that were non-gentle handled and fed RAC. The pH was not affected by handling for those pig handled gently. L-carnitine had no effect ($P < 0.20$) on pH.

A trend for an L-carnitine × handling interaction ($P < 0.09$) was observed for blood glucose concentration post-handling and the difference between pre-handling and post-handling blood glucose. Pigs that were non-gentle handled had higher post-handling glucose concentration and a greater difference (increase in glucose) between pre-handling and post-handling. Adding

L-carnitine to the diet further increased blood glucose in pigs in the non-gentle handling treatment but had no impact on blood glucose for pigs handled gently.

There was no effect of dietary treatment ($P > 0.28$) on pre-handling or post-handling urea N concentration. However, pigs that were non-gentle handled had a greater difference (greater increase; $P < 0.01$) in urea N concentration between pre-handling and post-handling.

Pigs fed RAC had higher ($P < 0.03$) pre-handling PCO_2 concentration compared to pigs not fed RAC. There was no effect ($P > 0.18$) of treatment on PCO_2 concentration post-handling or for the difference between pre-handling and post-handling. L-carnitine had no effect ($P = 0.29$) on PCO_2 concentration.

There was an L-carnitine × handling interaction ($P < 0.05$) for the difference in PO_2 concentration between pre-handling and post-handling. Pigs that were non-gentle handled had greater differences between pre-handling and post-handling PO_2 concentration compared to pigs that were gentle handled. Pigs fed L-carnitine and non-gentle handled had the highest PO_2 concentration difference compared to pigs that were not fed L-carnitine and non-gentle handled.

Pigs fed RAC tended ($P < 0.09$) to have higher pre-handling SO_2 concentration compared to pigs not fed RAC. There was no effect of treatment on post-handling SO_2 concentration or the difference between pre-handling and post-handling SO_2 concentration.

There was a RAC × handling interaction ($P < 0.04$) for the difference in HCO_3^- concentration between pre-handling and post-handling. RAC did not affect ($P > 0.68$) HCO_3^- concentration pre-handling. However, pigs that were non-gentle handled and/or fed RAC had decreased ($P< 0.01$) HCO_3^- concentration post-handling compared to pigs that were gentle handled and/or not fed RAC. The decrease in HCO_3^- concentration post-handling caused the interaction for the difference between pre-handling and post-handling, where HCO_3^- concentration was lower for pigs that were non-gentle handled compared to pigs that were gentle handled and lowest for pigs that were non-gentle handled and fed RAC compared with to pigs not fed RAC.

A RAC × handling interaction ($P < 0.01$) was observed for post-handling Na^+ concentration and a RAC × handling interaction ($P < 0.06$) trend was observed for the difference between pre-handling and post-handling Na^+ concentration. A RAC × handling interaction ($P < 0.01$) also was observed for post-handling K^+ concentration and for the difference between pre-handling and post-handling K^+ concentration. Pigs that were non-gentle handled had increased

post-handling Na^+ and K^+ concentration and an increased difference between pre-handling and post-handling Na^+ and K^+ concentration compared to pigs that were gentle handled and it increased further for pigs that were non-gentle handled and fed RAC compared to pigs that were not fed RAC.

Pigs fed RAC had lower ($P < 0.02$) pre-handling Cl^- concentration compared to pigs not fed RAC. Pigs that were non-gentle handled had ($P < 0.01$) higher post-handling Cl^- concentration and a greater difference (increase) in Cl^- concentration between pre-handling and post-handling compared to pigs that were handled gentle. Pigs that were fed RAC had a greater ($P < 0.02$) increase in Cl^- concentration when comparing pre-handling and post-handling values.

Pigs fed RAC had ($P < 0.01$) higher pre-handling Ca^{++} concentration and tended ($P < 0.07$) to have higher post-handling Ca^{++} concentration compared to pigs not fed RAC. Handling and L-carnitine did not influence ($P > 0.72$) Ca^{++} concentration.

There was a trend ($P < 0.07$) for a RAC × L-carnitine interaction for pre-handling Mg^{++} concentration. Adding RAC to the diet decreased Mg^{++} concentration in pigs not fed L-carnitine but had no effect in pigs fed L-carnitine.

A post-handling RAC × handling interaction ($P < 0.04$) was observed for post-handling cortisol concentration. Pigs that were non-gentle handled had increased post-handling cortisol concentration compared to pigs that were gentle handled. Feeding RAC only increased cortisol concentration when pigs were non-gentle handled. Adding L-carnitine tended to reduce ($P < 0.09$) cortisol and the increase in cortisol caused by the handling treatment with the greatest difference in gentle handled pigs (interaction, $P < 0.10$).

A RAC × handling interaction ($P < 0.06$) trend was observed for the difference in rectal temperature between pre-handling and post-handling. Pigs that that were handled non-gentle had higher post-handling rectal temperature and the difference between pre-handling and post-handling was greater for pigs that were handled non-gentle compared to gentle. There was a trend ($P < 0.06$) for pigs fed RAC to have higher post-handling rectal temperature compared to pigs that were not fed RAC. Pigs that were non-gentle handled and fed RAC had the highest increase in rectal temperature compared to pigs fed the other treatment diets. Pigs fed added L-carnitine had a lower ($P < 0.01$) pre-handling rectal temperature and a tendency for a greater ($P < 0.07$) difference (increase) between pre- and post-handling.

Pigs fed RAC tended ($P < 0.11$) to have higher minimum and average heart rate during the handling period compared to pigs not fed RAC (Table 4). Pigs that were non-gentle handled had increased ($P < 0.01$) average, maximum, and change in heart rate compared to pigs that were handled gentle. L-carnitine had no effect ($P > 0.15$) on heart rate.

Experiment 2. A trend ($P < 0.08$) for an L-carnitine × handling interaction was observed for post-handling LDH concentration (Table 5). Pigs fed L-carnitine and gentle handled had lower LDH concentration compared to pigs not fed L-carnitine and gentle handled; however, pigs fed L-carnitine and non-gentle handled had higher LDH concentration compared to pigs not fed L-carnitine and non-gentle handled. Pigs fed RAC had higher ($P < 0.01$) post-handling and 1 hr post-handling LDH concentration compared to pigs not fed RAC. Pigs that were non-gentle handled had higher ($P < 0.01$) post-handling LDH concentration and the difference between immediately post-handling and 1 hr post-handling was greater ($P < 0.01$) for pigs that were non-gentle handled compared to pigs that were handled gently.

Pigs that were non-gentle handled or fed RAC had higher ($P < 0.05$) post-handling lactate concentration compared to pigs gentle handled or not fed RAC. There was a trend ($P < 0.07$) for a RAC × handling and an L-carnitine × handling interaction for 1 h post-handling lactate concentration. Pigs that were non-gentle handled had higher lactate concentration 1 h post-handling compared to pigs gentle handled and it was higher in non-gentle handled pigs fed RAC or L-carnitine compared to pigs not fed RAC or L-carnitine. The difference between post-handling and 1 h post-handling lactate concentration was greater ($P < 0.01$) for pigs that were non-gentle compared to gentle handled. The difference (greater decrease) was greater because post-handling lactate concentration was much higher for pigs that were non-gentle handled compared to gentle handled and therefore had further to decrease to approach normal levels as the pig recovered from the non-gentle handling.

Post-handling pH was lower ($P < 0.02$) for pigs that were non-gentle handled or fed RAC compared to pigs that were gentle handled or not fed RAC. The pH of pigs that were non-gentle handled was still lower ($P < 0.03$) 1 h post-handling compared to pigs that were handled gentle. A trend was observed for a RAC × handling interaction ($P < 0.08$) for the difference between pH measured post-handling and 1 h post-handling. Pigs that were non-gentle handled or fed RAC had lower post-handling pH; therefore, the difference was greater for pigs that were non-gentle handled or fed RAC between post-handling and 1 h post-handling. Feeding pigs L-carnitine had

no effect on pH immediately or 1 h post-handling, but there was a trend ($P < 0.08$) for an L-carnitine × RAC interaction for the difference between the two. Adding either L-carnitine or RAC increased the difference between immediately and 1 h post-handling pH, but the response was not additive.

Pigs that were non-gentle handled had higher ($P < 0.01$) post-handling glucose concentration compared to pigs that were gentle handled. Pigs fed RAC had lower ($P < 0.04$) glucose concentration post-handling compared to pigs not fed RAC. A trend ($P < 0.07$) was observed for a RAC × L-carnitine interaction for glucose concentration 1 h post-handling. Pigs fed RAC had lower glucose concentration 1 h post-handling compared to pigs not fed RAC and pigs fed L-carnitine had lower glucose concentration 1 h post-handling compared to pigs that were not fed L-carnitine. Glucose concentration was lowest 1 h post-handling for pigs fed RAC and L-carnitine. A trend was observed for an L-carnitine × handling interaction ($P < 0.09$) for glucose concentration 1 h post-handling. Pigs that were non-gentle handled had lower glucose concentration 1 h post-handling compared to pigs that were gentle handled. Pigs fed L-carnitine and gentle handled had increased glucose concentration compared to pigs that were not fed L-carnitine and gentle handled; however, pigs fed L-carnitine and non-gentle handled had decreased glucose concentration compared to pigs that were not fed L-carnitine and non-gentle handled. The difference between post-handling and 1 h post-handling glucose concentration was ($P < 0.01$) greater (higher decrease) for pigs that were non-gentle handled compared to pigs that were gentle handled.

A RAC × L-carnitine × handling interaction ($P < 0.04$) was observed for post-handling and 1 h post-handling urea N concentration. Pigs fed either RAC or L-carnitine had decreased urea N concentrations and it was lowest for pigs that were handled gentle. Pigs that were non-gentle handled had higher post-handling and 1 h post-handling urea N concentration compared to pigs that were gentle handled. Pigs that were fed RAC or L-carnitine had lower urea N concentration post-handling and 1 h post-handling compared to pigs that were not fed RAC or L-carnitine. The difference between post-handling and 1 h post-handling urea N concentration was less ($P < 0.01$) for pigs that were non-gentle handled compared to pigs that were gentle handled.

Pigs that were fed L-carnitine had lower ($P < 0.05$) PCO_2 concentration post-handling compared to pigs not fed L-carnitine. Pigs that were non-gentle handled or fed RAC had ($P < 0.03$) lower PCO_2 concentration 1 h post-handling compared to pigs that were gentle handled or

not fed RAC. The difference between post-handling and 1 h post-handling PCO_2 concentration was ($P < 0.01$) greater (decreased more) for pigs that were non-gentle handled compared to pigs that were gentle handled.

Pigs that were non-gentle handled had higher ($P < 0.01$) post-handling PO_2 concentration compared to pigs that were gentle handled. Pigs fed L-carnitine had higher ($P < 0.03$) PO_2 concentration compared to pigs not fed L-carnitine. The difference between post-handling and 1 h post-handling PO_2 concentration was ($P < 0.03$) greater (bigger decrease) for pigs that were non-gentle handled compared to pigs that were gentle handled.

A trend was observed for pigs fed L-carnitine to have higher ($P < 0.07$) post-handling SO_2 concentration compared to pigs not fed L-carnitine. There was no effect ($P > 0.12$) of treatment on 1 h post-handling SO_2 concentration or the difference between post-handling and 1 h post-handling levels.

Pigs that were non-gentle handled had ($P < 0.01$) lower HCO_3^- concentration post-handling and 1 h post-handling compared to pigs that were gentle handled. Pigs fed either RAC or L-carnitine had lower ($P < 0.02$) post-handling HCO_3^- concentration and tended to have lower ($P < 0.08$) HCO_3^- concentration 1 h post-handling compared to pigs not fed RAC or L-carnitine.

Pigs that were non-gentle handled or fed RAC had higher ($P < 0.01$) post-handling Na^+ concentration compared to pigs that were gentle handled or not fed RAC. A RAC × L-carnitine × handling interaction ($P < 0.04$) was observed for 1 h post-handling Na^+ concentration, although the differences were minor. Na^+ was highest for pigs non-gentle handled and was decreased for pigs fed RAC and no L-carnitine; however, it increased when pigs were fed L-carnitine. Pigs that were non-gentle handled had higher Na^+ concentration 1 h post-handling compared to pigs that were gentle handled. Pigs that were non-gentle handled had the highest Na^+ concentrations with the levels decreasing when RAC was fed to pigs without L-carnitine in the diet, but increasing when RAC and L-carnitine were added to the diet together. Pigs that were non-gentle handled had ($P < 0.01$) higher K^+ concentration post-handling and pigs that were fed RAC tended to have ($P < 0.09$) higher K^+ concentration post-handling compared to pigs that were gentle handled or not fed RAC. A RAC × L-carnitine × handling interaction ($P < 0.02$) was observed for K^+ concentration 1 h post-handling, similar to the Na^+ interaction being minor in nature. Pigs that were non-gentle handled had increased K^+ compared to pigs gentle handled and the increase was greater for pigs fed RAC; however, it was decreased for pigs fed L-carnitine and handled non-

gentle. Pigs that were non-gentle handled had higher K^+ concentration than pigs that were handled gentle. Pigs that were non-gentle handled and fed RAC or L-carnitine had lower K^+ concentration compared to pigs that were non-gentle handled and not fed RAC or L-carnitine.

Pigs that were non-gentle handled had ($P < 0.01$) higher Cl^- concentration post-handling and pigs fed RAC tended ($P < 0.09$) to have higher Cl^- concentration post-handling compared to pigs gentle handled or not fed RAC. A RAC × L-carnitine interaction ($P < 0.04$) was observed for Cl^- concentration 1 h post-handling. Pigs fed either RAC or L-carnitine had lower Cl^- concentration 1 h post-handling compared to pigs not fed RAC or L-carnitine; however, pigs fed both RAC and L-carnitine had higher Cl^- concentration compared to pigs not fed L-carnitine and RAC. Pigs that were non-gentle handled had a greater ($P < 0.01$) difference (decrease) between Cl^- concentration post-handling and 1 h post-handling compared to pigs that were gentle handled.

A trend was observed ($P < 0.06$) for a RAC × handling interaction for post-handling Ca^{++} concentration. Pigs that were gentle handled and fed RAC had lower Ca^{++} concentration compared to pigs gentle handled and not fed RAC. However, pigs non-gentle handled and fed RAC had higher Ca^{++} concentration compared to pigs non-gentle handled and not fed RAC. A RAC × L-carnitine × handling interaction ($P < 0.05$) was observed for 1 h post-handling Ca^{++} concentration. Pigs that were non-gentle handled had lower Ca^{++} concentration compared to pigs that were gentle handled. Pigs fed either RAC or L-carnitine and non-gentle handled had lower Ca^{++} concentration compared to pigs not fed RAC or L-carnitine and non-gentle handled. Pigs handled non-gentle or fed RAC had a greater ($P < 0.04$) difference (decrease) between post-handling and 1 h post-handling Ca^{++} concentration compared to pigs that were gentle handled or not fed RAC.

Pigs that were non-gentle handled had ($P < 0.01$) higher Mg^{++} concentration post-handling, 1 h post-handling, and a had a greater ($P < 0.01$) difference (decrease) in Mg^{++} concentration between post-handling and 1 h post-handling compared to pigs that were gentle handled.

A RAC × L-carnitine × handling interaction trend ($P < 0.07$) was observed for post-handling cortisol concentration. Pigs that were non-gentle handled had higher post-handling cortisol concentration compared to pigs gentle handled. Pigs fed RAC or L-carnitine had increased cortisol concentration compared to pigs not fed RAC or L-carnitine. Post-handling cortisol concentration was highest for pigs fed RAC and L-carnitine and non-gentle handled.

Pigs that were non-gentle handled had ($P < 0.01$) higher 1 h post-handling cortisol concentration and a higher ($P < 0.01$) difference (increase) in cortisol concentration between post-handling and 1 h post-handling compared to pigs that were gentle handled.

A RAC × L-carnitine interaction ($P < 0.02$) was observed for post-handling rectal temperature and a RAC × L-carnitine trend ($P < 0.06$) was observed for 1 h post-handling rectal temperature. Pigs fed RAC had higher rectal temperature compared to pigs not fed RAC; however, it was highest for pigs fed RAC and L-carnitine compared to pigs fed RAC and not fed L-carnitine. Pigs that were non-gentle handled had higher ($P < 0.01$) rectal temperature post-handling and 1 h post-handling compared to pigs gentle handled.

Discussion

In evaluating growth performance, our results are in agreement with previous findings observing improvements in growth performance of pigs fed RAC (Armstrong et al., 2004; See et al., 2004; Apple et al., 2008). However we did not see improvements in gain or feed efficiency with added dietary L-carnitine, whereas in some studies improvements have been observed (James et al., 2009a). Some of the differences between the present study and earlier trials may be a result of location of the experiments. Some of the previous experiments were conducted in a commercial finishing research facility where environment and space allowance generally reduce feed intake approximately 30% compared to pigs reared in university research facilities (De la Llata et al., 2001).

Lactate dehydrogenase is a cytoplasmic enzyme that catalyzes a reversible reaction which converts pyruvate to lactate at the end of anaerobic glycolysis. There are several isoenzymes of LDH. However, isoenzyme analysis requires special assays that are not widely available; therefore in our experiments we analyzed total LDH. An increase in LDH is an indicator of muscle damage and hemolysis (Stockham and Scott, 2002). Increased LDH activity may be due to local or diffuse cell damage. Pigs that were non-gentle handled had greater LDH immediately post-handling compared to pigs that were handled gentle. Although LDH concentration increased between pre-handling and post-handling for pigs gentle handled, the magnitude was minor compared to the non-gentle handled pigs. This is just one of the criteria involved which demonstrate that the handling course was successful in eliciting differences between gentle and non-gentle handled pigs. Pigs that were fed RAC were more susceptible to an increase in LDH

due to both handling treatments. They also had greater LDH 1 h after handling which indicates a longer return to normal for pigs fed RAC than in pigs not fed RAC. Dietary L-carnitine has been shown to increase pyruvate carboxylase and decrease LDH in pigs (Owen et al., 2001). An increase in pyruvate carboxylase may direct pyruvate away from lactate, thus reducing substrate available for lactic acid synthesis. Furthermore, a decrease in LDH may delay the onset of glycolysis. However, in this experiment, added L-carnitine did not alleviate the production of LDH produced in pigs that were non-gentle handled or fed RAC.

Benjamin et al. (2001) and Peterson et al. (2009) demonstrated that pigs aggressively handled had higher serum lactate compared to pigs gentle handled. This is consistent with reports from Anderson et al. (2002) and Ivers et al. (2002a, b) who observed that pigs handled aggressively had higher lactate concentrations and that these were positively correlated with downer or non-ambulatory pigs. These reports are in agreement with our observations. Within 1 h post-handling, lactate concentrations were still elevated compared to pigs that were gentle handled. This illustrates the importance of allowing pigs ample time to recover after delivery to slaughter facilities so that the increased lactate concentrations do not affect meat quality. It is of interest that pigs fed RAC had increased levels of pre-handling lactate compared to pigs not fed RAC. This may suggest that pigs fed RAC were in a partial acidotic state before handling. Pigs that were fed RAC also had increased post-handling lactate concentrations compared to pigs not fed RAC. Pigs that were fed RAC and non-gentle handled had the greatest lactate concentrations and it remained greater 1 h post-handling. We did not observe differences in LDH for pigs fed added L-carnitine and lactate concentrations were not affected.

Downer pigs have been reported to have decreased blood pH (Anderson et al., 2002; Ivers et al., 2002a, b). Pre-handling pH was decreased in pigs fed RAC compared to pigs not fed RAC. This supports the observation that pre-handling lactate concentrations were increased for pigs fed RAC and may simply be a description of lactate level and acid-base balance. Non-gentle handling of pigs in our experiment decreased post-handling pH compared to pigs gentle handled and it was lowest for pigs fed RAC suggesting that RAC amplifies the affect of non-gentle handling and that pigs were in a state of metabolic acidosis. Peterson et al. (2009) observed decreased pH in pigs that were aggressively handled compared to pigs gentle handled; however, pH was not different for pigs fed RAC compared to pigs not fed RAC in their experiment. Pigs fed RAC did not have different pH 1 h post-handling compared to pigs not fed RAC. Although

pH was still decreased 1 h post-handling for pigs non-gentle handled, it was near levels of pigs that were gentle handled. In comparison, lactate levels were still almost 5-fold higher 1 h post-handling for pigs non-gentle handled compared to gentle. This demonstrates the magnitude of importance that the pig places on maintaining acid-base balance. Bertol et al. (2002) reported that pigs fed L-carnitine had reduced changes in blood pH when subjected to vigorous handling procedures and electrical prod stimulation. This is in contrast to the observations from our experiments in which pH was not affected post-handling or 1 h post-handling by feeding L-carnitine. A trend was observed for a RAC × L-carnitine interaction in the change in pH between post-handling and 1 h post-handling. Pigs fed L-carnitine in combination with RAC tended to have increased pH (better recovery) within 1 h post-handling compared to pigs not fed L-carnitine.

Anderson et al. (2002) and Benjamin et al. (2001) have reported that pigs handled aggressively have increased blood glucose concentrations compared to pigs gently handled. Glucose concentrations increase from the breakdown of glycogen under stress and are caused by stimulation of the stress hormones epinephrine and norepinephrine. In agreement, we observed increased glucose concentrations post-handling in pigs that were non-gentle handled compared to gentle. In Exp. 2, pigs fed RAC tended to have decreased post-handling glucose concentration. Pigs fed RAC or L-carnitine had decreased 1 h post-handling glucose concentration and it was lowest for pigs fed both RAC and L-carnitine. In comparison, Bertol et al. (2005) observed that pigs fed L-carnitine had lower baseline glucose values compared to pigs not fed L-carnitine. This may have been a result of pigs fed L-carnitine being more able to use added fat for energy through accelerated fatty acid oxidation and increased β-oxidation (Owen et al., 2001).

In our first experiment, pigs that were non-gentle handled had a greater change (increase) in urea N concentration between pre-handling and post-handling concentrations. In Exp. 2, pigs that were non-gentle handled also had increased urea N concentrations. This may be the result of increased muscle breakdown occurring from the stress of non-gentle handling. However, pigs fed either RAC or L-carnitine had decreased post-handling and 1 h post-handling urea N concentrations. Urea N concentrations would be expected to be low in pigs fed RAC because of the increased protein deposition (Webster et al., 2007); however, Rincker et al. (2003) observed no difference in urea N in weanling pigs fed added L-carnitine.

An increase in PCO_2 (partial pressure of CO_2) is an indicator of hypercapnia and results from excess CO_2 in blood, whereas a decrease in PCO_2 is an indicator of hypocapnia and results from a deficiency of CO_2 in blood. The normal pulmonary process is related to acid-base balance. The expiration of CO_2 results in elimination of H^+. Because blood $[H^+]$ is very low compared to $[HCO_3^-]$ (ratio ≈ 1:600,000), this process does not lower $[HCO_3^-]$ unless there is excessive generation of H^+ (Stockham and Scott, 2002). Anderson et al. (2002) and Ivers et al. (2002b) have reported that downer pigs had decreased PCO_2 compared to non-downer pigs. In Exp. 1, pigs fed RAC had increased pre-handling PCO_2 concentration compared to pigs not fed RAC. Whereas in Exp. 2, pigs fed L-carnitine had decreased PCO_2 immediately post-handling compared to pigs not fed L-carnitine. Pigs that were non-gentle handled or fed RAC had decreased PCO_2 1 h post-handling compared to pigs that were handled gentle or not fed RAC. In metabolic acidosis, increased $[H^+]$ stimulates respiration and the result is increased removal of CO_2 from pulmonary blood which in turn decreases PCO_2. This supports that pigs that were non-gentle handled or fed RAC were in a state of metabolic acidosis 1 h post-handling and that added L-carnitine did not alleviate the effects of stress.

In our experiments, we measured PO_2 in venous blood (partial pressure of O_2) and SO_2 (percent Hgb saturated with O_2 in arterial blood); however, arterial blood is the preferred sample for the assessment of oxygenation of the blood by the respiratory system. Decreased PO_2 (partial pressure of oxygen) is an indicator of hypoxemia which results from deficiency of dissolved O_2 in blood. However, post-handling PO_2 was greater for pigs that were non-gentle handled probably as a result of increased respiration rate compared to pigs that were gentle handled. Post-handling PO_2 was also greater for pigs fed L-carnitine in Exp. 2 and for non-gentle handled pigs in Exp. 1. In contrast, Bertol et al. (2005) reported that changes in blood PO_2 levels were less for pigs fed 5% soy oil and 150 ppm L-carnitine after a standard handling procedure than pigs not fed added soy oil and L-carnitine.

The SO_2 percentage is the amount of O_2 in blood divided by the O_2 carrying capacity of blood (expressed as a percentage). Bertol et al. (2005) observed that pigs fed L-carnitine had a decreased change between baseline and post-handling SO_2 percentage compared to pigs not fed L-carnitine. We observed that pigs fed L-carnitine had increased SO_2 post-handling (Exp. 2) compared to pigs not fed L-carnitine and were thus able to carry more O_2 in the blood. Pigs fed

RAC had decreased pre-handling SO_2 (Exp. 1) compared to pigs not fed RAC. The amount of O_2 available to transport in the blood was less for pigs fed RAC.

Several respiratory and non-respiratory processes help maintain $[H^+]$ at a stable concentration. Metabolic processes continually produce H^+ and it is either excreted (via kidneys) or bound to buffers (HCO_3^-, PO_4, NH_3, sulfates, Hgb, and other proteins such as albumin). Of the total buffering capacity, HCO_3^- contributes over 20 mmol/L whereas the non-bicarbonate buffers contribute less than 10 mmol/L (Stockham and Scott, 2002). Anderson et al. (2002) and Ivers et al. (2002b) reported that downer pigs have decreased HCO_3^- compared to non-downer pigs. Ivers et al. (2002b) investigated the effect of dietary cation-anion difference (DCAD = meq ($Na^+ + K^+ - Cl^-$)) on stress responses and downer pig incidence. In their study, pigs were fed either a high DCAD (HD, +481 meq/kg) or a low DCAD (LD, +81 meq/kg) diet. The authors observed fewer downer pigs fed HD than LD and HCO_3^- was greater for pigs fed HD compared to LD. Excess [H+] in metabolic acidosis leads to consumption or decreased concentration of HCO_3^- which is used as a buffer. In our experiments HCO_3^- was decreased post-handling for pigs that were non-gentle handled or fed RAC (Exp.1 and 2), or fed L-carnitine (Exp. 2). The concentration of HCO_3^- was still decreased 1 h post-handling for pigs that were non-gentle handled, fed RAC, or fed L-carnitine; however, they had increased from immediately post-handling concentrations. This demonstrates that the pigs were recovering from the handling treatment. Added L-carnitine did not affect the difference between pre- and post-handling HCO_3^- concentration and indicates that added L-carnitine did not alleviate or speed up the recovery of pigs that were non-gentle handled or fed RAC.

Strong cations and anions in biologic fluids are involved acid-base balance. Strong cations or strong anions are those ions that are completely dissociated in physiologic fluids. Strong cations (Na^+, K^+, Ca^{++}, Mg^{++}) are considered bases because when added to extracellular fluid, if there is not a balancing shift of a strong ion (e.g., remove K^+ or add Cl^-), then H^+ shifts out of the extracellular fluid to make it more alkaline. Strong anions (Cl^-, SO_4, lactate, acetoacetate, β-hydroxybutyrate, and other acidic products of metabolism) are considered acids because when added to extracellular fluid, if there is not a balancing shift of a strong ion (e.g., add Na^+ or remove lactate), then H^+ shifts into the extracellular fluid to make it more acidic (Stockham and Scott, 2002). Acidosis is a condition where there is an excess of strong anions or a deficit of strong cations. Anderson et al. (2002) observed that downer pigs had increased Na^+,

Ca^{++}, and K^{+} and decreased base excess compared to non-downer pigs. This is supported by observations from Ivers et al. (2002a,b). Our results are similar in that non-gentle handled pigs had increased Na^{+}, and decreased Cl^{-} (Exp. 1), and increased Na^{+}, K^{+}, and Ca^{++} immediately post-handling compared to pigs that were gentle handled. This demonstrates the impact that non-gentle handling of pigs has on the acid-base balance of pigs. Pigs fed RAC were more susceptible to changes in cation-anion concentration when they were non-gentle handled which suggests that non-gentle handling of pigs fed RAC causes metabolic acidosis.

Hypercortisolemia is a result of stress caused by an illness, trauma, environmental changes which stimulate cortisol releasing hormone, then adrenocorticotrophic hormone (corticotropin), and thus stimulates the adrenal glands to produce more cortisol (Stockham and Scott, 2002). Short stressful events (i.e., direct handling, isolation, and transportation) are usually followed by an increase in stress hormones (von Borell, 2001). Pigs that become downers have increased cortisol levels compared to non-downer pigs (Anderson et al., 2002; Ivers et al., 2002a, b). To avoid causing stress and biasing the data, when blood was collected, pigs were snared and released within less than approximately 30 s. It has been reported indirectly that snaring pigs and releasing them quickly does not affect cortisol concentrations (Haussmann et al., 2000). Similar to other stress response criteria, pigs that were non-gentle handled in our study had increased levels of cortisol and it was increased further for pigs that were fed RAC (Exp. 1). Sutherland et al. (2006) measured plasma cortisol as an indicator of hypothalamic-pituitary-adrenal (HPA) axis activation in response to 14 d of heat stress, restricted floor space, and social tension due to mixing of pigs at d 0. In their study, plasma cortisol was suppressed at d 7 and 14 in stressed pigs compared with non-stressed pigs. Their observation was likely due to a decrease in the responsiveness of, and even habituation of, the HPA axis to stress. Cortisol concentrations generally peak at approximately 30 min after initiation of stress in pigs (Prunier et al., 2005). Cortisol activity increases blood glucose concentrations by stimulating gluconeogenesis and creating a state of insulin resistance (Stockham and Scott, 2002). This may partially explain the increase in glucose concentrations that we observed in pigs that were non-gentle handled compared to gentle handled. This is supported by Anderson et al. (2002) and Benjamin et al. (2002) who also observed increased glucose concentrations in pigs that were aggressively handled compared to pigs that were gentle handled.

Pigs subjected to aggressive handling and use of electric prodding had increased skin temperature (Benjamin et al., 2001) and rectal temperature (Peterson et al., 2009). Brundige et al. (1998) evaluated the effect of using hurdles or electric prods to load pigs onto a trailer and observed that pigs shocked with an electric prod had higher rectal temperature 15 min post-loading than hurdle loaded pigs. In our experiment, pre-handling rectal temperature was slightly lower for pigs fed L-carnitine; however, it is difficult to explain a mechanism for this observation. Pigs that were non-gentle handled had increased rectal temperature immediate post-handling (Exp. 1 and 2) and 1 h post-handling (Exp. 2) compared to pigs that were gentle handled. Pigs that were fed RAC also had increased post-handling and 1 h post-handling rectal temperature (Exp. 2) compared to pigs not fed RAC and it was highest for pigs fed RAC in combination with L-carnitine. These results also indicate that our model was effective in demonstrating stress response differences between the two handling treatments and pigs fed RAC.

Aggressive handling of pigs and use of electric prodding has been reported to increase heart rate compared to gentle-handling (Benjamin et al., 2001). Marchant-Forde et al. (2003) demonstrated that feeding pigs RAC affected behavior, heart rate and catecholamine profile and made them more difficult to handle and potentially more susceptible to handling and transport stress. In our experiment, minimum heart rate was not affected by any of the treatments. Furthermore, we did not observe an affect of dietary treatment on any of the heart rate measurements. However, pigs that were non-gentle handled had increased average, maximum, and change (maximum - minimum) in heart rate compared to pigs that were handled gentle.

These results demonstrate the importance of proper handling on the metabolic affects of stress. Pigs fed RAC are more susceptible to stress when aggressively handled compared to pigs not fed RAC. Dietary L-carnitine did not alleviate the effects of stress when fed in combination with RAC.

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Figure 4.1 Diagram of handling course. Each handling treatment consisted of moving pigs back and forth (3 laps) in the alleyway of the finishing barn. In the gentle handling treatment, the handler moved pigs through the 150 m course, including a 15 o split-race loading ramp, using a sorting board at a moderate pace. In the non-gentle handling treatment, pigs were moved at a quicker pace through the 150 m course, including a 30 o single chute loading ramp and panels were used to narrow the alleyway to stimulate crowding. Pigs were subjected to three (one-second) stimulations, by an electrical prod, per lap around the course.

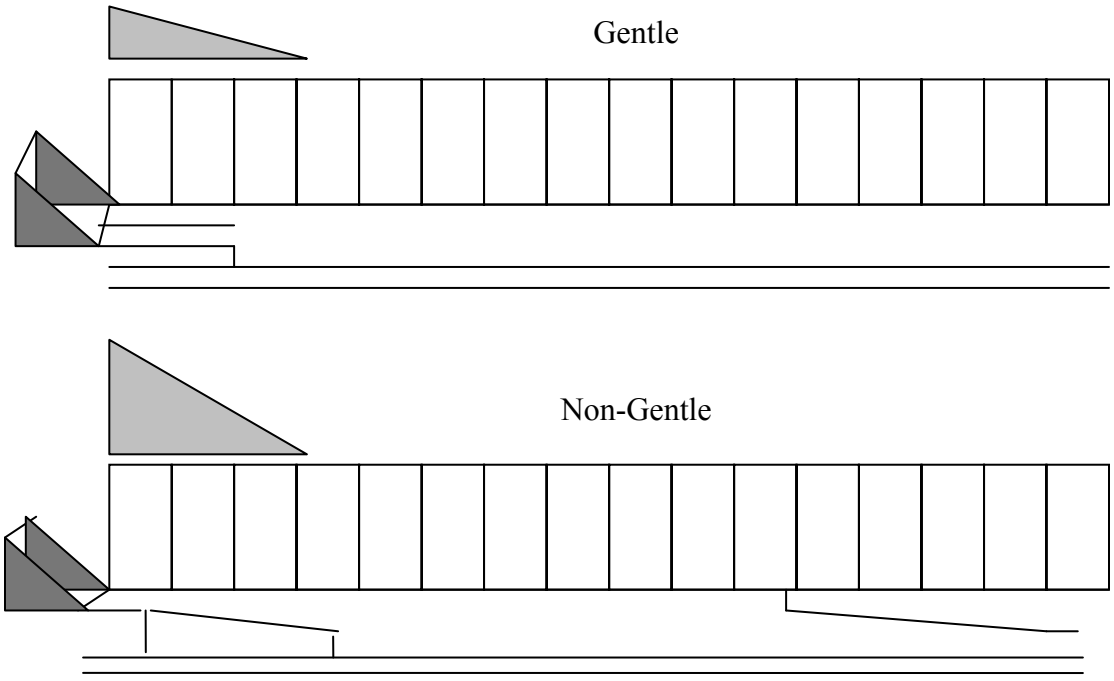

Table 4.1 Basal diet composition (Exp. 1 and 2, as-fed basis)[a]

Ingredient, %	Phase I [b]	Phase II [b]	Phase III [b]
Corn	66.92	74.26	74.45
Soybean meal (46.5% CP)	30.07	22.82	22.80
Monocalcium phosphate, 21% P	1.15	1.10	0.90
Limestone	0.96	0.93	0.90
Salt	0.35	0.35	0.35
Vitamin premix[c]	0.15	0.15	0.15
Trace mineral premix[d]	0.15	0.15	0.15
Medication[e]	0.05	0.05	-
Corn starch[f]	0.05	0.05	0.15
L-Lysine·HCl	0.15	0.15	0.15
Calculated composition			
CP (N × 6.25), %	19.67	16.92	16.92
Lysine, %	1.20	1.00	1.00
Lysine:calorie ratio, g/mcal	3.18	2.65	2.20
ME, kcal/kg	3,311	3,318	3,325
Ca, %	0.70	0.65	0.61
P, %	0.64	0.60	0.55

[a]Diets were formulated to meet or exceed NRC (1998) requirements.
[b]Phase I (36 to 54 kg, BW); Phase II (54 to 86 kg, BW); Phase III (86 to 118 kg, BW)
[c]Vitamin premix provided (per kilogram of complete diet): vitamin A, 6,614 IU; vitamin D_3, 992 IU; vitamin E, 26.5 IU; menadione (menadione dimethylpyrimidinol bisulphite), 2.65; vitamin B_{12}, 0.03 mg; riboflavin, 5.95 mg; pantothenic acid, 19.8 mg; and niacin, 33.1 mg.
[d]Trace mineral premix provided (per kilogram of complete diet): Mn (from manganese oxide), 39.7 mg; Fe (from ferrous sulfate), 165.3 mg; Zn (from zinc oxide) 165.3 mg; Cu (from copper sulfate), 16.5 mg; I (from calcium iodate) 0.3 mg; and Se (from sodium selenite), 0.3 mg.
[e]Provided 44 mg tylosin per kg diet.
[f]L-carnitine replaced cornstarch to provide either 0 or 50 ppm carnitine in Phase I, II, and III. RAC replaced cornstarch to provide either 0 or 20 ppm ractopamine·HCl in Phase III.

Table 4.2 Combined interactive effects between L-carnitine and Ractopamine-HC1 on growth performance of finishing pigs in Exp. 1 and 2[a]

	L-carnitine, ppm							
	0		50					
	Ractopamine HCl, ppm					Probability ($P <$)		
Item	0	20	0	20	SED	L-carnitine × Ractopamine HCl	L-carnitine	Ractopamine HCl
Pre-ractopamine HCl								
ADG, kg	0.96	-	0.94	-	0.02	-	0.40	-
ADFI, kg	2.48	-	2.48	-	0.03	-	0.95	-
G:F	0.39	-	0.38	-	0.01	-	0.45	-
Day 0 to 28								
ADG, kg	0.88	1.00	0.87	1.05	0.03	0.28	0.58	0.01
ADFI, kg	2.45	2.38	2.58	2.31	0.16	0.53	0.86	0.31
G:F	0.37	0.43	0.34	0.46	0.03	0.30	0.94	0.01
Overall								
ADG, kg	0.93	0.97	0.93	0.97	0.01	0.83	0.76	0.01
ADFI, kg	2.45	2.41	2.48	2.40	0.06	0.72	0.88	0.36
G:F	0.38	0.40	0.37	0.41	0.01	0.53	0.68	0.01

[a]Values are means eight observations(pens) and 12 pigs per pen.

Table 4.3 Interactive effects of L-carnitine, Ractopamine HC1, and handling on stress parameters of finishing pigs (Exp. 1)[a]

	Handling															
	Gentle				Non-gentle					Probability ($P <$)						
	L-carnitine, ppm															
	0		50		0		50			L-carnitine × Ractopamine	L-carnitine × Ractopamine	Ractopamine HCl × L-carnitine	Ractopamine HCl × handling	Ractopamine		
	Ractopamine HCl, ppm															
Item	0	20	0	20	0	20	0	20	SED	HCl × handling	HCl	× handling	handling	L-carnitine	HCl	Handling
LDH, U/L																
Pre-handling	533	533	537	534	550	604	558	594	25.7	-	0.76	-	-	0.95	0.46	-
Post-handling	488.	5885	574.	600	651	775	648	769	38	0.58	0.55	0.39	0.35	0.48	0.01	0.01
Difference	-45	55	37	66	101	171	90	175	28	0.51	0.69	0.34	0.89	0.41	0.01	0.01
Lactate, mmol/L																
Pre-handling	2.39	3.61	2.23	2.31	2.10	2.85	2.03	2.91	0.26	-	0.35	-	-	0.17	0.01	-
Post-handling	4.70	5.93	5.08	5.85	19.38	21.39	19.16	27.51	1.67	0.21	0.28	0.30	0.13	0.26	0.03	0.01
Difference	2.31	2.32	2.85	3.54	17.28	18.54	17.13	24.60	1.63	0.35	0.99	0.11	0.09	0.03	0.29	0.01
pH																
Pre-handling	7.39	7.37	7.40	7.40	7.41	7.43	7.40	7.39	0.01	-	0.81	-	-	0.20	0.04	-
Post-handling	7.41	7.39	7.41	7.38	7.20	7.11	7.22	7.05	0.02	0.32	0.29	0.71	0.01	0.60	0.01	0.01
Difference	0.02	0.02	0.01	-0.02	-0.21	-0.32	-0.18	-0.34	0.03	0.61	0.37	0.99	0.05	0.33	0.01	0.01
Glucose, mg/dL																
Pre-handling	87.25	88.38	88.50	89.75	87.88	84.25	82.50	88.25	1.82	-	0.20	-	-	0.86	0.54	-
Post-handling	92.00	84.50	90.00	88.13	128.25	122.13	138.13	149.00	5.02	0.57	0.27	0.09	0.49	0.06	0.82	0.01
Difference	4.75	-3.88	1.50	-1.62	40.37	37.88	55.63	60.75	5.37	0.92	0.54	0.08	0.51	0.09	0.67	0.01
Urea nitrogen, mg/dL																
Pre-handling	15.75	13.63	15.13	15.63	15.00	12.38	13.38	12.75	1.13	-	0.31	-	-	0.98	0.29	
Post-handling	15.88	13.63	15.50	15.88	16.38	13.88	14.88	14.13	1.17	0.85	0.36	0.51	0.77	0.89	0.28	0.73
Difference	0.13	0	0.37	0.25	1.38	1.50	1.50	1.38	0.20	0.34	0.75	0.75	1.00	0.11	0.52	0.01
PCO_2, mmHg																
Pre-handling	62.75	64.26	59.50	62.03	58.94	61.59	57.19	62.86	1.40	-	0.48	-	-	0.29	0.03	-
Post-handling	56.31	56.05	55.38	57.50	50.10	55.40	50.26	52.46	3.10	0.46	0.92	0.66	0.45	0.76	0.21	0.18
Difference	-6.44	-8.21	-4.12	-4.53	-8.84	-6.19	-6.93	-10.40	3.54	0.41	0.60	0.37	0.88	0.69	0.74	0.53
PO_2, mmHg																
Pre-handling	40.31	40.00	44.78	38.64	53.19	49.05	40.04	41.30	3.90	-	0.98	-	-	0.26	0.55	-
Post-handling	39.35	54.14	39.14	39.50	59.29	48.39	52.89	62.75	6.02	0.14	0.79	0.34	0.49	0.77	0.55	0.04
Difference	-0.96	14.14	-5.64	0.86	6.10	-0.66	12.85	21.45	7.12	0.30	0.77	0.05	0.40	0.64	0.31	0.28

SO_2, %																
Pre-handling	68.93	67.18	76.04	64.59	76.68	72.18	71.36	70.29	2.72	-	0.57	-	-	0.81	0.09	-
Post-handling	66.64	68.74	69.38	67.25	73.18	64.74	76.50	65.00	4.20	0.93	0.61	0.87	0.16	0.73	0.16	0.66
Difference	-2.29	1.56	-6.66	2.66	-3.50	-7.44	5.14	-5.29	5.78	0.50	0.95	0.43	0.13	0.67	0.95	0.78
HCO_3, mmol/L																
Pre-handling	38.41	37.35	37.55	38.76	37.79	38.20	37.99	38.15	0.43	-	0.25	-	-	0.69	0.68	-
Post-handling	35.93	34.21	35.14	34.83	19.63	17.89	21.51	14.63	1.28	0.11	0.36	0.77	0.11	0.70	0.01	0.01
Difference	-2.48	-3.14	-2.41	-3.93	-18.16	-20.31	-16.48	-23.52	1.32	0.35	0.95	0.18	0.04	0.42	0.07	0.01
Na^+, mmol/L																
Pre-handling	147.38	147.50	146.75	146.38	145.50	147.25	146.63	147.25	0.45	-	0.37	-	-	0.73	0.25	-
Post-handling	147.88	147.88	148.50	146.75	151.00	154.63	151.75	153.50	0.78	0.96	0.15	0.96	0.01	0.72	0.15	0.01
Difference	0.50	0.38	1.75	0.37	5.50	7.38	5.12	6.25	0.59	0.83	0.40	0.25	0.06	0.92	0.53	0.01
K^+, mmol/L																
Pre-handling	5.00	5.09	4.93	4.96	5.15	5.00	4.84	5.25	0.09	-	0.17	-	-	0.48	0.30	-
Post-handling	5.03	4.96	5.08	4.95	4.81	5.68	4.73	5.63	0.13	0.82	0.95	0.68	0.01	0.82	0.01	0.12
Difference	0.03	-0.13	0.15	-0.01	-0.34	0.68	-0.11	0.38	0.16	0.34	0.32	0.56	0.01	0.76	0.03	0.39
Cl^-, mmol/L																
Pre-handling	104.25	102.75	103.88	102.75	103.50	102.50	102.88	102.63	0.39	-	0.47	-	-	0.57	0.02	-
Post-handling	104.38	103.88	104.13	104.25	108.38	108.63	108.75	109.75	0.69	0.95	0.51	0.51	0.43	0.43	0.67	0.01
Difference	0.13	1.13	0.25	1.50	4.88	6.13	5.87	7.12	0.57	0.90	0.90	0.47	0.90	0.23	0.02	0.01
Ca^{++}, mg/dL																
Pre-handling	5.71	5.94	5.81	5.79	5.64	5.73	5.63	5.79	0.05	-	0.38	-	-	0.96	0.01	-
Post-handling	5.58	5.66	5.57	5.54	5.53	5.63	5.50	5.71	0.10	0.24	0.98	0.34	0.18	0.72	0.07	0.96
Difference	-0.13	-0.28	-0.24	-0.25	-0.11	-0.10	-0.13	-0.08	0.09	0.66	0.49	0.71	0.34	0.80	0.61	0.19
Mg^{++}, mg/dL																
Pre-handling	0.97	0.93	0.90	0.92	0.95	0.92	0.93	0.91	0.01	-	0.07	-	-	0.02	0.03	-
Post-handling	0.94	0.92	0.91	0.92	2.18	1.06	0.99	1.07	0.31	0.33	0.30	0.34	0.38	0.31	0.38	0.19
Difference	-0.03	-0.01	0.01	0	1.23	0.14	0.06	0.16	0.30	0.31	0.33	0.33	0.40	0.35	0.42	0.19
Cortisol, ng/ml																
Pre-handling	12.45	14.81	14.15	9.92	15.99	18.36	12.93	15.11	1.73	-	0.33	-	-	0.18	0.70	-
Post-handling	42.85	46.21	36.20	34.03	49.48	60.86	48.15	61.68	5.07	0.49	0.76	0.10	0.04	0.08	0.02	0.01
Difference	30.40	31.39	22.05	21.98	33.49	42.49	35.22	46.57	4.10	0.83	089	0.13	0.21	0.09	0.15	0.01
Temperature, °C																
Pre-handling	39.17	39.29	38.99	39.04	39.40	39.44	39.16	39.18	0.13	-	0.78	-	-	0.01	0.49	-
Post-handling	40.00	40.08	40.00	40.00	40.99	41.33	40.91	41.24	0.18	0.86	0.80	0.80	0.14	0.50	0.06	0.01
Difference	0.83	0.79	1.01	0.96	1.60	1.89	1.75	2.06	0.17	0.94	1.00	0.94	0.06	0.07	0.16	0.01

[a]Values are means of 8 observations (pigs). There were 2 pigs per pen per handling group.

Table 4.4 Interactive effects of L-carnitine, Ractopamine HC1, and handling on heart rate of finishing pigs (Exp. 1)

	Handling									Probability ($P <$)						
	Gentle				Non-gentle											
	L-carnitine, ppm															
	0		50		0		50			L-carnitine × Ractopamine						
	Ractopamine HCl, ppm										L-carnitine × Ractopamine			Ractopamine		
Item	0	20	0	20	0	20	0	20	SED	HCl × handling	HCl	× handling	HCl × handling	L-carnitine	HCl	Handling
Heart rate																
Minimum	118	114	121	132	118	137	118	123	12.53	0.18	0.99	0.11	0.38	0.75	0.11	0.73
Average	192	184	193	200	204	210	230	217	11.14	0.09	0.82	0.19	0.09	0.56	0.11	0.01
Maximum	251	247	258	264	279	281	275	289	10.79	0.93	0.22	0.28	0.42	0.15	0.35	0.01
Change (max-min)	133	133	138	132	164	141	153	167	13.19	0.10	0.20	0.66	0.92	0.46	0.56	0.01
Observations/trt	6	8	5	7	6	6	4	4								

Table 4.5 Interactive effects of L-carnitine, Ractopamine HC1, and handling on stress parameters of finishing pigs (Exp. 2)[a]

	Handling									Probability ($P <$)						
	Gentle				Non-gentle											
	L-carnitine, ppm															
	0		50		0		50			L-carnitine × Ractopamine						
	Ractopamine HCl, ppm										L-carnitine ×			Ractopamine		
Item	0	20	0	20	0	20	0	20	SED	HCl × handling	Ractopamine HCl	L-carnitine × handling	HCl × handling	L-carnitine	Ractopamine HCl	Handling
LDH, U/L																
Post-handling	476	621	457	532	509	560	542	637	29.5	0.23	0.69	0.08	0.41	0.86	0.01	0.13
1hr Post-handling	463	588	451	529	600	624	594	708	28.1	0.15	0.66	0.12	0.49	0.93	0.01	0.01
Difference	5	-33	-6	-3	91	63	52	71	19.7	0.94	0.28	0.53	0.74	0.88	0.58	0.01
Lactate, mmol/L																
Post-handling	2.78	5.94	4.10	5.08	19.38	20.43	18.90	22.24	2.36	0.29	0.98	0.84	0.95	0.67	0.05	0.01
1hr Post-handling	2.61	2.73	2.89	2.29	9.54	10.23	10.25	14.50	1.84	0.13	0.31	0.07	0.06	0.09	0.12	0.01
Difference	-0.16	-3.21	-1.21	-2.79	-9.84	-10.20	-8.65	-7.74	1.99	0.96	0.51	0.31	0.22	0.47	0.33	0.01
pH																
Post-handling	7.46	7.42	7.44	7.43	7.13	7.07	7.10	7.03	0.04	0.56	0.74	0.50	0.33	0.43	0.02	0.01
1hr Post-handling	7.42	7.44	7.43	7.44	7.38	7.40	7.38	7.33	0.02	0.42	0.27	0.25	0.49	0.36	0.96	0.03
Difference	-0.04	0.02	-0.01	0.00	0.25	0.34	0.27	0.30	0.02	0.89	0.08	0.66	0.57	0.98	0.01	0.01
Glucose, mg/dL																
Post-handling	84.25	72.38	86.38	80.88	168.88	149.63	156.63	152.63	10.43	0.70	0.35	0.39	0.80	0.95	0.09	0.01
1hr Post-handling	88.25	78.25	86.25	81.00	100.38	76.63	73.13	75.75	4.21	0.21	0.07	0.09	0.73	0.11	0.04	0.64
Difference	4.00	5.88	-0.13	0.13	-68.50	-73.00	-83.50	-76.88	10.48	0.57	0.67	0.69	1.00	0.20	0.85	0.01
Urea nitrogen, mg/dL																
Post-handling	14.75	13.13	13.50	11.88	20.25	12.25	15.38	13.38	0.87	0.04	0.04	0.65	0.02	0.03	0.01	0.03
1hr Post-handling	15.50	13.75	14.38	12.75	21.00	12.25	14.88	13.50	0.87	0.01	0.01	0.19	0.02	0.01	0.01	0.18
Difference	0.75	0.63	1.38	0.88	0.75	0.00	-0.50	0.13	0.26	0.11	0.35	0.07	0.64	0.81	0.48	0.01
PCO_2, mmHg																
Post-handling	49.33	50.70	49.09	47.81	50.19	51.01	49.21	40.61	1.79	0.35	0.10	0.26	0.28	0.05	0.29	0.41
1hr Post-handling	57.35	53.28	54.24	52.24	46.06	43.75	46.08	38.14	2.67	0.27	0.65	0.81	0.52	0.19	0.03	0.01
Difference	8.03	2.58	5.15	4.73	-4.13	-7.36	-3.14	-2.48	3.61	0.91	0.38	0.51	0.74	0.61	0.40	0.01
PO_2, mmHg																
Post-handling	38.25	39.81	55.21	40.29	51.19	55.96	65.56	72.10	5.47	0.41	0.50	0.55	0.27	0.03	0.93	0.01
1hr Post-handling	42.63	45.50	40.15	40.30	38.38	49.08	41.51	50.50	3.64	0.94	0.76	0.41	0.26	0.83	0.13	0.46
Difference	4.38	5.69	-15.06	0.01	-12.81	-6.89	-24.05	-21.60	6.61	0.52	0.70	0.97	0.76	0.06	0.35	0.03

SO_2, %																
Post-handling	71.00	70.71	80.38	73.14	68.35	68.74	71.91	76.78	3.33	0.37	0.85	0.99	0.32	0.07	0.86	0.48
1hr Post-handling	68.39	74.61	71.31	72.90	64.61	74.34	71.74	73.95	3.27	0.82	0.33	0.66	0.74	0.53	0.12	0.84
Difference	-2.61	3.90	-9.06	-0.24	-3.74	5.60	-0.18	-2.83	4.84	0.40	0.56	0.73	0.61	0.36	0.19	0.72
HCO_3, mmol/L																
Post-handling	35.44	33.10	33.61	32.20	17.54	15.14	15.84	11.00	1.28	0.36	0.68	0.40	0.34	0.02	0.01	0.01
1hr Post-handling	36.05	36.06	36.06	35.51	22.81	27.90	27.80	21.51	2.01	0.27	0.18	0.12	0.13	0.07	0.08	0.01
Difference	0.61	2.96	2.45	3.31	11.28	12.76	11.96	10.51	1.28	0.73	0.30	0.38	0.46	0.88	0.45	0.01
Na^+, mmol/L																
Post-handling	145.38	147.13	145.50	146.50	152.25	153.50	151.50	155.75	1.11	0.11	0.34	0.39	0.24	0.67	0.01	0.01
1hr Post-handling	145.00	145.63	145.38	145.75	147.75	145.50	146.00	147.00	0.74	0.04	0.07	0.64	0.17	0.88	0.88	0.13
Difference	-0.38	-1.50	-0.13	-0.75	-4.50	-8.00	-5.50	-8.75	0.88	0.91	0.72	0.20	0.02	0.72	0.01	0.01
K^+, mmol/L																
Post-handling	4.73	4.90	4.79	4.70	5.15	5.55	5.40	5.78	0.19	0.63	0.56	0.22	0.17	0.50	0.09	0.01
1hr Post-handling	4.83	5.06	4.98	4.94	5.18	5.34	4.69	4.61	0.15	0.02	0.25	0.57	0.04	0.66	0.01	0.09
Difference	0.10	0.16	0.19	0.24	0.03	-0.21	-0.71	-0.16	0.18	0.07	0.08	0.06	0.65	0.23	0.33	0.02
Cl^-, mmol/L																
Post-handling	103.38	103.75	102.75	103.50	109.75	110.50	109.63	111.00	0.90	0.89	0.60	0.51	0.60	0.79	0.09	0.01
1hr Post-handling	102.63	102.63	102.00	102.88	104.13	102.88	102.88	104.25	0.60	0.29	0.04	0.76	0.65	0.88	0.54	0.10
Difference	-0.75	-1.13	-0.75	-0.63	-5.63	-7.63	-6.75	-6.75	0.74	0.33	0.11	0.62	0.26	0.87	0.15	0.01
Ca^{++}, mg/dL																
Post-handling	5.34	5.29	5.36	5.29	5.56	5.69	5.61	5.66	0.09	0.69	0.48	0.99	0.06	0.88	0.66	0.01
1hr Post-handling	5.58	5.44	5.55	5.54	5.30	5.27	5.43	5.08	0.08	0.05	0.40	0.53	0.31	0.98	0.02	0.01
Difference	0.24	0.14	0.19	0.25	-0.26	-0.42	-0.18	-0.58	0.09	0.17	0.78	0.62	0.07	0.95	0.04	0.01
Mg^{++}, mg/dL																
Post-handling	1.00	0.98	0.96	0.97	1.12	1.14	1.13	1.14	0.03	0.63	0.86	0.33	0.61	0.72	0.69	0.01
1hr Post-handling	1.05	1.00	1.00	1.01	1.11	1.07	1.06	1.09	0.03	0.80	0.18	0.96	0.70	0.46	0.61	0.01
Difference	0.05	0.01	0.04	0.03	0.00	-0.07	-0.08	-0.05	0.02	0.45	0.11	0.43	0.96	0.51	0.30	0.01
Cortisol, ng/ml																
Post-handling	34.46	38.48	38.42	40.16	42.11	37.92	42.90	56.03	3.85	0.07	0.16	0.21	0.76	0.02	0.17	0.08
1hr Post-handling	20.99	32.12	19.47	25.33	58.74	59.48	61.18	69.49	6.37	0.42	0.89	0.20	0.62	0.80	0.11	0.01
Difference	-13.47	-6.35	-18.95	-14.83	16.63	21.56	18.27	13.46	6.63	0.61	0.34	0.57	0.41	0.13	0.40	0.01
Temperature, °C																
Post-handling	40.30	40.47	40.17	40.63	41.03	41.02	40.88	41.46	0.15	0.42	0.02	0.51	0.87	0.40	0.01	0.01
1hr Post-handling	39.45	39.67	39.31	39.71	40.44	40.30	39.84	40.56	0.21	0.20	0.06	0.67	0.95	0.41	0.03	0.01
Difference	-0.85	-0.79	-0.85	-0.93	-0.60	-0.72	-1.04	-0.90	0.19	0.39	0.76	0.30	0.95	0.10	1.00	0.83

[a]Values are means of 8 observations (pigs). There were 2 pigs per pen per handling group.

CHAPTER 5 - Effect of Phytase Dosage and Source on Growth Performance and Bone Development of Nursery Pigs

Abstract

A 28-d growth assay was conducted to determine the effect of phytase dosage and source on growth performance of nursery pigs (initially 10.6 kg). The nine experimental treatments were control diets (0.13, 0.18, and 0.23% available P) and phytase (100, 225, or 350 FTU or FYT/kg) from either Natuphos® or Ronozyme™ P added to the diet containing 0.13% available P. Increasing available P increased (linear, $P < 0.01$) ADG, ADFI, and G:F. No phytase source × level interactions ($P > 0.14$) or differences between phytase sources ($P > 0.27$) were observed. Increasing phytase increased (linear, $P < 0.01$) ADG and G:F. Increasing available P increased (linear, $P < 0.01$) bone bending moment. Increasing available P or phytase increased (linear, $P < 0.02$) percentage bone ash with no difference ($P = 0.89$) between phytase sources. Regression analysis of the ADG response indicated that, when adding 350 phytase units/kg or less, each 100 phytase units/kg will release 0.022 and 0.017% available P for Natuphos® and Ronozyme™ P, respectively. Regression analysis of bone bending moment and percentage bone ash indicated that each 100 phytase units/kg will release 0.022 and 0.023% available P for Natuphos® and 0.015 and 0.020% available P for Ronozyme™ P, respectively. The results of this experiment indicate that increasing available P or phytase level, through 0.23% available P and 350 FTU or FYT/kg, respectively, improves ADG, G:F, and bone criteria.

Key Words: Nursery Pigs, Phosphorus, Phytase

Introduction

Supplementing phytase in swine diets is becoming increasingly common as a method to improve the availability of phosphorus in plant ingredients containing high levels of phytate phosphorus. The improved availability provides a means to lower the amount of phosphorus in diets (Jendza et al., 2006; Augspurger et al., 2007; Olukosi et al., 2007), and thus may contribute a greater economic return. The addition of phytase to swine diets has also been shown to decrease phosphorus excretion by as much as 44 to 61% (Grandhi, 2001; Veum et al., 2006).

The environmental benefits associated with using phytase are becoming more important as many states are changing nutrient plans from a nitrogen basis to a phosphorus basis. Natuphos®, a product of Aspergillus niger, (BASF Corporation, Florham Park, NJ) and Ronozyme™ P (CT), (Roche, Parsippany, NJ) produced from Peniophoria lycii are two available phytase sources. Natuphos® is a 3-phytase enzyme that initiates dephosphorylation at the 3-phosphate position on the phytate inositol ring, and Ronozyme™ P is a 6-phytase that initiates the dephosphorylation of phytate at the 6-phosphate position (BASF, 2000). Augspurger et al. (2003, 2004) observed greater P release in chicks fed Natuphos®, compared with those fed Ronozyme™ P. Therefore, the objective of this experiment was to determine if Natuphos® and Ronozyme™ P have equal effects on growth performance and bone development of the growing pig.

Materials and Methods

General

Procedures used in these experiments were approved by the Kansas State University Animal Care and Use Committee. A pilot study initially was conducted with increasing levels of available P. The results of the pilot study were used in formulating our basal diet to contain 0.13% available P to demonstrate a linear response to increasing levels of available phosphorus in the experiment. The basal diet was corn-soybean meal-based and was formulated to contain 5% added fat, 1.3% total lysine, and 0.13% available P (Table 1). All other nutrients were formulated to exceed the recommended requirements (NRC, 1998). Diets were fed in meal form.

A total of 342 barrows (Line 42 sire × C22 dam; PIC, Franklin, KY) were used in the 28-d growth assay. Pigs were weaned at approximately 18 d of age and fed a three phase starter program from d 0 to 17 post-weaning (Tokach et al., 1997). All diets contained 0.45% and 0.36% available phosphorus. From d 17 to 20 after weaning, pigs were fed a common diet without inorganic P (0.10% available P) to ensure a response to increasing P in the experiment. On d 20 post-weaning (10.6 kg BW), pigs were blocked by weight and allotted randomly to nine dietary treatments in a randomized complete block design. Each treatment had eight replications and four or five pigs per pen (there were the same number of pigs in a pen for each block).

Monocalcium phosphate was substituted for sand to form the other control diets (0.18 and 0.23% available P). Phytase (100, 225, or 350 FTU or FYT/kg) from either Natuphos® or

Ronozyme™ P was added to the diet containing 0.13% available P at the expense of sand. Calcium to total P ratio was maintained at 1.12:1 in all diets, similar to NRC (1998) estimates. All ingredients providing either Ca or P to the diet were analyzed for Ca and P by using inductively coupled plasma emission spectroscopy with a Fisons ARL model 3410 (Ecublens, Switzerland; AOAC, 1995, Method 985.01), and analyzed values were used in diet formulation. Phytase from Natuphos® and Ronozyme™ P also was analyzed before diet formulation to equalize actual phytase percentage in the experimental diets. Diets were analyzed for phytase after the conclusion of the experiment at both BASF and Roche. The analyzed values for the three levels of phytase (100, 225, and 350 FYT/kg or FTU/kg) were 138, 337, and 427 FYT/kg and 104, 144, and 324 FTU/kg for Ronozyme™ P and Natuphos®, respectively.

Pigs were housed in an environmentally controlled nursery. Each pen (1.2 m^2) contained a stainless steel self-feeder and one nipple waterer to allow ad libitum access to feed and water. Pigs and feeders were weighed every seven d during the experiment to determine ADG, ADFI, and G:F.

At the conclusion of the 28-d experiment, one pig per pen (closest to average weight of all pigs in the pen) was transported to the Kansas State University College of Veterinary Medicine and euthanized via captive bolt. The left femur, 3rd and 4th metatarsal bones, and 5th, 6th, and 7th ribs were collected, labeled, and placed in plastic bags until further evaluation. Samples were stored at –29°C. Bones were removed from the freezer, separated, cleaned of all connective tissue, labeled, and stored in individual bags at –12 °C. The 6th rib was used to obtain all rib data.

Bones were then measured for mechanical properties (bending moment) by using a three-point flexure test (Crenshaw et al., 1981) with force applied by an Instron Universal Testing Machine model 4201 (Instron Corp., Canton, MA). Crosshead speed was 100 mm/min. Ribs and metatarsals were oriented to the cross head such that the force applied was medial-lateral, whereas the femurs were oriented such that force was applied dorsal-ventral. The distance between the two fulcra points for ribs and metatarsals was 2 cm and the distance for the femur was 3 cm.

After the mechanical tests were performed, the 6th rib, and 3rd and 4th metatarsals were cut in half with a model 5215 Hobard meat saw (Hobart Corp., Troy, Ohio) with a blade that was 0.32 cm thick. Bones were cut adjacent to the point at which the force was applied to obtain bone

dimensions. Bones were placed in petroleum ether for 7 d, and then dried for 24 h at 105°C to determine the absolute dry, fat-free weight. The 6th rib, and 3rd and 4th metatarsals were then heated to 600 °C to determine percentage bone ash. Ash is expressed as a percentage of dried, fat-free bone weight.

Statistical Analyses

Growth performance data were analyzed in a randomized complete-block design according to the PROC MIXED procedures of SAS, with pen as the experimental unit. Linear and quadratic polynomial contrasts were performed to determine the effects of increasing amounts of available phosphorus and phytase. Contrasts were performed to compare phytase sources. A regression analysis of the ADG response was conducted by plotting the improvement in ADG with each incremental increase (0.05 and 0.10%) in available P, compared with that of the control diet containing 0.13% available P. The percentage of available P that was released was calculated by comparing the ADG curve of each source of phytase with that of the controls. A repeated-measures analysis was used for analysis of the bone criteria (Littell et al., 1996) according to the PROC MIXED procedures of SAS. The model included the fixed effects of treatment × bone interaction, with the random effects of block and repeated measures of bone within pig. Linear and quadratic contrasts were used to further characterize treatment effects.

Results and Discussion

Increasing available P increased (linear, $P < 0.01$) ADG, ADFI, and G:F over the length of the 28-d experiment (Table 2). There were no phytase source × level interactions ($P > 0.14$). There were no differences between phytase sources ($P > 0.27$) observed for ADG or G:F, but ADFI tended to be greater ($P = 0.09$) for those pigs fed Natuphos® compared with Ronozyme™ P. Increasing phytase increased (linear, $P < 0.01$) ADG and G:F from d 0 to 28. Feed intake increased (quadratic, $P < 0.05$) with increasing phytase.

Increasing available P increased (linear, $P < 0.01$) bending moment. There was a phytase source × bone interaction ($P < 0.04$) for bending moment (Table 3). The femur, 3rd metatarsal, and 4th metatarsal had increased bending moments with increasing levels of Ronozyme™ P or Natuphos®, but the 6th rib did not demonstrate the same response.

There were no phytase source × bone interactions ($P > 0.05$) for percentage of bone ash. Increasing available P increased (linear, $P < 0.01$) percentage of bone ash. Increasing phytase from either Natuphos® or Ronozyme™ P increased (linear, $P < 0.01$) percentage of bone ash, with no difference ($P > 0.89$) between phytase sources.

Regression analysis of the ADG response (Figure 1) indicated that, when adding 350 phytase units/kg or less, each 100 phytase units/kg will release 0.022 and 0.017% available P for Natuphos® and Ronozyme™ P, respectively.

Regression analysis of the main effects of bending moment (Table 4) indicated that, when adding less than 350 phytase units/kg, each 100 phytase units/kg would release 0.021 and 0.023% available P for Natuphos® and Ronozyme™ P, respectively. The same calculations for percentage of bone ash indicated that each 100 phytase units/kg would release 0.015 and 0.020% available P for Natuphos® and Ronozyme™ P, respectively.

Natuphos® is a recombinant enzyme from *Aspergillus niger*, whereas Ronozyme™ P is a recombinant enzyme from *Peniophoria lycii* (Augspurger et al., 2004; Veum et al., 2006). Natuphos® and Ronozyme™ P initiate dephosphorylation of phytate complexes at either the 3- or the 6-phytase position, respectively (Augspurger et al., 2003; Veum et al., 2006). The pH optima for phytase activity is bimodal for Natuphos® (pH 2.5 and 5.5) and Ronozyme™ P (pH 4.0 to 4.5). Because of the differences between the two phytase sources, the objective of this experiment was to determine if Natuphos® and Ronozyme™ P have equal effects on growth performance and bone development of the growing pig.

Results from this experiment demonstrated that ADG, ADFI, and G:F increased linearly over the range of available P levels fed to pigs (0.13, 0.18, and 0.23% available phosphorus). This suggests that the basal diet (0.13% available P) was deficient in available P and that pigs responded to added inorganic P from monocalcium phosphate. These results are supported by Sands et al. (2001) and Jendza et al. (2006), who demonstrated linear improvements in growth performance to increasing levels of inorganic P from monosodium phosphate in 10-kg pigs.

Pigs fed increasing levels of phytase added to the basal diet had linearly improved ADG and G:F, but pigs fed the highest concentration of phytase (350 FTU or FYT/kg) from either Natuphos® or Ronozyme™ P did not have as large an ADG or G:F, compared with those of pigs fed the diet formulated to 0.23% available P. These results suggest that phytase from either

source was able to release a portion of the phytate-bound P from the basal diet. This data is consistent with observed responses in pigs receiving phytase-supplemented diets from Natuphos® (Zhang et al., 2000; Omogbenigun et al., 2003; Kies et al., 2006). In our experiment, there were no significant differences between phytase sources for ADG or G:F, although those fed Natuphos® tended to have greater ADFI than those fed Ronozyme™ P. There have been relatively few comparison studies of Natuphos® and Ronozyme™ P that have been conducted with pigs. Augspurger et al. (2003) reported that chicks fed 500 and 1,000 FTU/kg Natuphos® (0.37% total P basal diet) gained more than chicks fed 500 and 1,000 FYT/kg from Ronozyme™ P in one experiment, but found no differences between sources in a second experiment. The authors conducted a pig trial with added phytase (400 FTU or FYT/kg to a 0.34% total P basal diet) from both sources and, although Natuphos® numerically had better ADG and G:F, compared with Ronozyme™ P, the difference was not significant because there was more variability in the pig trial than in the chick trial. These results support our data, which indicated that pigs fed Natuphos® had numerically greater ADG than did pigs fed Ronozyme™ P.

Measurement of bending moment is a flexure test, which involves both compressive and tensile forces. Bending represents the type of force (compressive and tensile), and moment is the product of force and distance. Bending moment is the force applied to the bone, adjusted for the distance (length) over which it is applied, and is measured in units of force and distance (kilograms-centimeters). Calculation of bending moment allows comparisons between bones of different lengths (Crenshaw et al., 1981). In our experiment, pigs fed increasing amounts of available P from monocalcium phosphate had a linear increase in bone bending moment. These results agree with the growth performance data and suggest that bending moment is a good indicator of inorganic P bioavailability. We did observe a treatment × bone interaction for bending moment. Pigs fed increasing levels of either Ronozyme™ P or Natuphos® had increasing bending moments for the femur, 3rd metatarsal, and 4th metatarsal. Increasing levels of available P or phytase increased the percentage of bone ash. There were no differences in percentage of bone ash between Ronozyme™ P or Natuphos® in our experiment. In general, bone ash measurements are more responsive to dietary phosphorus than growth performance criteria, and bone mechanical properties are even better indicators of phosphorus concentration than percentage of bone ash (Zhang et al., 2000; Jendza et al., 2006; Veum et al., 2006).

Maximization of growth and efficiency of feed utilization occur at dietary calcium and P levels less than those required to maximize bone criteria (Crenshaw, 1986). The dietary concentration of P and calcium that results in maximal bone ash content and bone strength is 0.10 percentage unit higher than that resulting in maximal growth performance (NRC, 1998). The combination of growth and bone measurements from our experiment should have detected differences between the two phytase sources if they were not equal in efficacy.

Although significant differences were not observed between the two phytase sources, an analysis of the ADG and G:F response indicated that, when adding less than 350 phytase units/kg, each 100 phytase units will release 0.022 and 0.024% available P for Natuphos®, and 0.017 and 0.033% available P for Ronozyme™ P, respectively. Analysis for bending moment and percentage bone ash indicated that 100 phytase units/kg would release 0.021 and 0.015% available P for Natuphos® and 0.023 and 0.20% available P for Ronozyme™ P, respectively.

Implications

Increasing available P or phytase concentration, through 0.23% available P and 350 FTU or FYT/kg phytase, improves ADG, G:F, and bone development of nursery pigs. When adding up to 350 FTU or FYT/kg from either Natuphos® or Ronozyme™ P to the diet, available P percentages can be reduced by approximately 0.01 to 0.02% for every 100 phytase units. The two sources of phytase released approximately the same amount of available P; therefore, decisions to use one source of phytase or the other should be based on economic and feed-manufacturing criteria.

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Figure 5.1 Regression of ADG to determine available P release from each unit of phytase. Values are means of eight replications (pens) and one pig per pen. Available P released for every 100 units of phytase was calculated by plotting a regression analysis of the response to additional increments of available P.

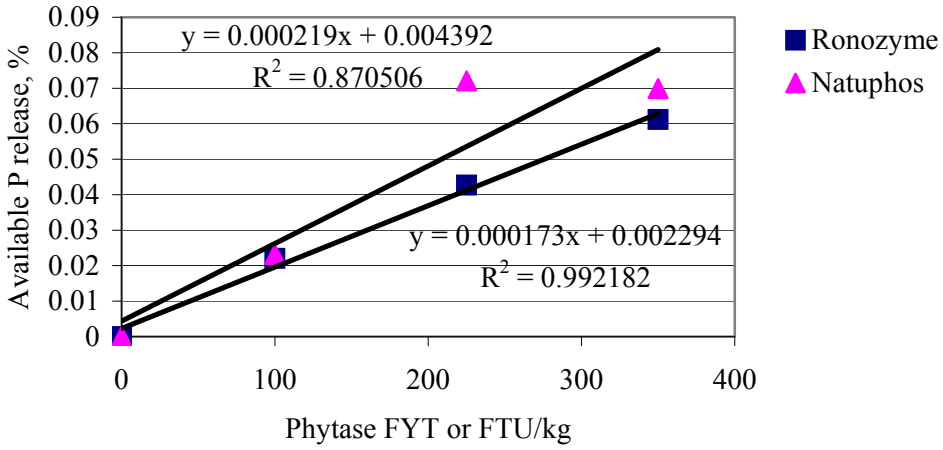

Table 5. 1 Basal diet composition (as-fed basis)[a]

	Available P, %		
Ingredient, %	0.13[b]	0.18	0.23
Corn	57.97	57.98	57.98
Soybean meal, 46.5% CP	34.15	34.15	34.15
Soybean oil	5.00	5.00	5.00
Sand	0.60	0.30	---
Limestone	0.52	0.56	0.60
Antimicrobial[c]	0.50	0.50	0.50
Salt	0.35	0.35	0.35
Monocalcium phosphate, 21% P	0.32	0.57	0.83
Vitamin premix[d]	0.25	0.25	0.25
Trace mineral premix[e]	0.15	0.15	0.15
L-Lysine·HCl	0.15	0.15	0.15
DL-Methionine	0.04	0.04	0.04
Calculated composition			
CP (N × 6.25), %	20.80	20.80	20.80
ME, kcal/kg	3,558	3,558	3,558
Ca, %	0.46	0.52	0.58
P, %	0.41	0.46	0.51
Available P, %	0.13	0.18	0.23
Lysine, %	1.30	1.30	1.30
Methionine, %	0.36	0.36	0.36
Threonine, %	0.81	0.81	0.81

[a]Analyzed Ca values used in formulation were: corn, 0.003%; soybean meal 0.29%; limestone, 36.68%; antimicrobial, 11.56%; monocalcium phosphate, 16.09%; vitamin premix, 16.83%; and trace mineral premix, 13.33%. Analyzed P values used in formulation were: corn, 0.21%; soybean meal, 0.66%; limestone, 0.002%; antimicrobial, 0.06%; monocalcium phosphate, 19.30%; vitamin premix, 0.03%; and trace mineral premix, 0.00%.

[b]Phytase from either Natuphos® or Ronozyme™ P was added to provide 100, 225, or 350 FTU or FYT/kg at the expense of sand.

[c]Provided 25 g/ton carbadox.

[d]Contributed per kilogram of complete diet: vitamin A, 8,181 IU; vitamin D_3, 1,322 IU; vitamin E, 35.27 IU; menadione (menadione dimethylpyrimidinol bisulphite), 3.52 mg; vitamin B_{12}, 0.03 mg; riboflavin, 7.94 mg; pantothenic acid, 26.46 mg; niacin, 44.10 mg; choline, 110.3 mg; biotin, 0.04 mg; folic acid, 0.33 mg; and pyridoxine, 3.03 mg.

[e]Contributed per kilogram of complete diet: Zn (from zinc oxide), 165.3 mg; Fe (from ferrous sulfate), 165.3 mg; Mn (from manganese oxide), 39.7 mg; Cu (from copper sulfate), 16.5 mg; I (from calcium iodate), 0.3 mg; and Se (from sodium selenite), 0.3 mg.

Table 5.2 Effect of available P and phytase source on growth performance of nursery pigs[a]

				Phytase source[b,c]											
				Ronozyme™ P,			Natuphos®,				Probability ($P <$)				
	Available P, %			FYT/kg			FTU/kg					Available P, %		Phytase level	
Item	0.13	0.18	0.23	100	225	350	100	225	350	SEM	Source[d]	Linear	Quadratic	Linear	Quadratic
Day 0 to 28															
ADG, g	602	650	694	623	642	659	624	669	667	12.23	0.27	0.01	0.86	0.01	0.28
ADFI, g	944	992	1032	936	973	979	961	1022	984	16.66	0.09	0.01	0.81	0.01	0.05
G:F	0.64	0.66	0.67	0.67	0.66	0.67	0.65	0.65	0.68	0.01	0.29	0.01	0.97	0.01	0.15

[a]Values are means of eight replications (pens) and four or five pigs per pen, initial BW, 10.6 kg.
[b]Diets were identical to treatment containing 0.13% available P with the exception of phytase.
[c]No phytase level × source interactions ($P > 0.14$).
[d] Comparison between Rhonozyme™ P vs Natuphos®.

Table 5.3 Effect of available P and phytase source on growth performance of nursery pigs[a]

	Phytase source[b]											Probability ($P <$)			
	Available P, %			Ronozyme™ P, FYT/kg			Natuphos®, FTU/kg					Available P, %		Phytase level	
Item	0.13	0.18	0.23	100	225	350	100	225	350	SED[c]	Source	Linear	Quadratic	Linear	Quadratic
Bending moment, kg-cm															
Femur	55.05	70.30	86.24	49.32	78.05	74.53	59.91	70.93	78.01	9.45	0.73	0.01	0.97	0.01	0.89
3rd Metatarsal	9.91	11.72	13.05	9.50	11.99	12.40	10.09	12.05	11.73	0.98	0.99	0.01	0.78	0.02	0.87
4th Metatarsal	8.96	11.25	12.09	9.04	11.41	11.15	9.24	11.71	11.36	0.87	0.64	0.01	0.25	0.01	0.38
6th Rib	3.15	4.01	6.66	3.19	3.78	3.99	2.98	3.82	3.98	0.76	0.89	0.01	0.18	0.11	0.87
Mean of all bones[e]	19.26	24.36	29.51	18.07	26.31	25.52	20.55	24.63	26.27	1.98	0.74	0.01	0.99	0.02	0.78
Ash, %															
3rd Metatarsal	39.05	40.18	43.07	38.33	40.05	40.76	39.67	41.26	40.93	1.10	0.44	0.01	0.36	0.04	0.64
4th Metatarsal	36.64	39.09	42.32	38.95	39.86	39.89	38.06	39.77	39.80	1.20	0.60	0.01	0.70	0.01	0.88
6th Rib	34.21	36.82	42.70	35.75	37.47	41.37	36.24	38.22	38.67	2.55	0.76	0.01	0.46	0.05	0.73
Mean of all bones	36.63	38.69	42.70	37.68	39.09	40.67	37.99	39.75	39.38	0.96	0.89	0.01	0.38	0.02	0.54

[a]Values are means of eight replications (pens) and one pig per pen. A repeated-measures analysis was used for analysis of the bone criteria. The model included the fixed effects of treatment × bone interaction, with the random effects of block and repeated measures of bone within pig.

[b]Diets were identical to treatment containing 0.13% available P with the exception of phytase.

[c]Standard error of the difference.

[d]Comparison between Rhonozyme™ P vs Natuphos®.

[e]Treatment × bone interaction ($P < 0.04$); no other interactions observed ($P > 0.10$).

Table 5.4 Calculated available P release for every 100 units of phytase[a,b]

	% Available P released Phytase source	
Item	Ronozyme™ P	Natuphos®
ADG	0.017	0.022
G:F	0.033	0.024
Bending moment, kg-cm	0.023	0.021
Ash, %	0.020	0.015
Bending moment, kg-cm		
Femur	0.024	0.022
3rd Metatarsal	0.027	0.020
4th Metatarsal	0.023	0.024
Ash, %		
4th Metatarsal	0.016	0.017

[a]Values are means of eight replications (pens) and one pig per pen.
[b]Available P released for every 100 units of phytase was calculated by forming a regression analysis of the response to each incremental increase (0.05 and 0.10%) in available P, compared with that of the control diet containing 0.13% available P.