Effects of oral administration of sodium citrate or acetate to pigs on blood parameters, postmortem glycolysis, muscle pH decline, and quality attributes of pork[1,2]

J. W. Stephens,*[3] M. E. Dikeman,*[4] J. A. Unruh,* M. D. Haub,† M. D. Tokach,* and S. S. Dritz‡

*Department of Animal Sciences and Industry, 226 Weber Hall, †Department of Human Nutrition, and ‡College of Veterinary Medicine, Kansas State University, Manhattan 66506

ABSTRACT: The objective of this study was to determine the effects of oral administration of sodium citrate (CIT) or acetate (ACE) to pigs on blood parameters, postmortem glycolysis, pH decline, and quality attributes of pork. Previous studies have shown that CIT has the potential to inhibit phosphofructokinase (PFK), a key enzyme in postmortem muscle glycolysis. In Exp. 1, CIT, ACE, or water was orally administered (0.75 g/kg of BW) to 24 pigs. After a 30-min rest, pigs were exercised, and blood samples were taken at 45 and 75 min after oral treatment. Citrate and ACE tended (P = 0.08) to increase blood pH and increased (P = 0.02) bicarbonate levels immediately after exercise. After a 30-min rest, blood pH of pigs administered ACE tended (P = 0.09) to remain higher, whereas blood pH of CIT-treated pigs was similar to that of control pigs. Bicarbonate levels in ACE- and CIT-treated pigs were still greater ($P < 0.05$) than those of control pigs at 75 min after oral treatment. In Exp. 2, 30 pigs were administered CIT, ACE, or water 45 min before stunning (electric plus captive bolt). Antemortem treatments had no effect ($P > 0.10$) on muscle pH or postmortem concentrations of the glycolytic metabolites of glucose-6 phosphate, fructose-6 phosphate, fructose-1,6 bisphosphate, glyceraldehyde-3 phosphate, dihydroxyacetone phosphate, or lactate. Minor, but inconsistent, differences in quality attributes were found in LM chops, and no differences in quality attributes were found between control and CIT- or ACE-treated pigs for inside and outside semimembranosus muscles ($P > 0.10$). There was no significant inhibition of the PFK enzyme by orally administered CIT or ACE; however, the PFK glycolytic metabolite data analysis indicated that PFK was a main regulatory enzyme in postmortem muscle.

Key words: pork, glycolysis, citrate, acetate, meat quality

J. Anim. Sci. 2008. 86:1669–1677
doi:10.2527/jas.2007-0797

INTRODUCTION

Visual attributes that define pork quality include color, firmness, wetness, and marbling. These attributes are of great economic importance because they affect consumer appeal, eating satisfaction, and repeat purchases. Muscle color, firmness, and wetness are highly dependent on the reactions of glycolysis and pH decline during the onset of rigor (Puolanne et al., 2002). Anaerobic glycolysis that occurs in postmortem muscle produces lactate and hydrogen ions. These hydrogen ions accumulate in muscle and reduce pH. If this reaction occurs at an accelerated rate before adequate chilling, the combination of low pH and high temperature has the potential to denature muscle proteins, resulting in pale color, softness, and diminished water-holding capacity (Sayre and Briskey, 1963).

Glycolysis is regulated by the enzyme phosphofructokinase (**PFK**) in postmortem muscle (Dalrymple and Hamm, 1975; Hamm, 1977; Rhoades et al., 2005). This enzyme catalyzes the reaction that transfers a phosphate group from ATP to fructose-6 phosphate (**F6P**) to form fructose-1,6 bisphosphate (**F16BP**) and ADP. Although ATP is a substrate for this reaction and is the source of the transferred phosphate, it also serves as an inhibitor of the enzyme (Kemp and Krebs, 1967). In live animals, a high content of ATP indicates excess energy; therefore, ATP inhibits PFK, slowing glycolysis

[1]Contribution No. 06-15-J of the Kansas State University Agricultural Experiment Station, Manhattan, KS.

[2]The authors wish to express their appreciation to S. B. Smith at Texas A&M University for consultation and assistance with glycolytic procedures used in this study.

[3]Current address: University of Arkansas, Fayetteville, AR 72701.

[4]Corresponding author: mdikeman@ksu.edu

Received December 12, 2007.
Accepted March 4, 2008.

and, ultimately, ATP production. Large amounts of ADP in the cell indicate a lack of energy; thus, ADP activates PFK.

Citrate is produced in the body from acetyl CoA and oxaloacetate as the first step of the aerobic citric acid cycle. Citrate has been shown to inhibit PFK in mammalian muscle (Newsholme et al., 1977). Citrate inhibits PFK by binding directly to PFK, which decreases the enzyme's affinity for its substrate, F6P, and its activator, ADP. Citrate also increases the enzyme's affinity for ATP at the substrate site and, more important, at the inhibitory site (Kemp and Krebs, 1967).

Exercise physiologists have used the blood-alkalizing and PFK-inhibitory properties of citrate to enhance athletic performance (Potteiger et al., 1996a,b; Linossier et al., 1997). These researchers stated that citrate has an alkalizing effect on blood, and may inhibit the formation of lactate and hydrogen ions associated with anaerobic glycolysis resulting from intense exercise bouts (Linossier et al., 1997). Fushimi et al. (2001) inhibited PFK activity in rats by feeding them sodium acetate 2 h before death. The researchers theorized that acetate was converted to citrate in the body by citrate synthase, and that citrate inhibited PFK. When injected directly into the muscle 50 min postmortem, citrate increased muscle pH but had no effect on pork quality attributes (Stephens et al., 2006). Therefore, the objectives of our study were to determine the effects of oral administration of sodium citrate or acetate to pigs on blood parameters, postmortem glycolysis, muscle pH decline, and quality attributes of pork.

MATERIALS AND METHODS

Pigs were cared for and handled according to procedures approved by the Kansas State University Institutional Animal Care and Use Committee.

Exp. 1

Two replications of 12 finishing pigs were fed a standard finishing diet, containing 20 mg/kg of ractopamine, for 14 d before the experiment, and then were fasted for no less than 20 h, and no greater than 26 h. Pigs were then blocked by BW into 8 groups of 3 and assigned to the citrate, acetate, or control treatment. Pigs were restrained by using a snout snare and were administered their antemortem treatments by placing a tube through the mouth and down the esophagus. Pigs assigned to the citrate treatment (**CIT**) and the acetate treatment (**ACE**) treatments were given 0.75 g/kg of BW of sodium citrate or sodium acetate in a 1:3 (wt/vol) solution of distilled water, followed by 60 mL of water to flush the tube. Control pigs were given 180 mL of distilled water. Pigs were allowed to rest for 30 min, and then were moved at a trotting pace up and down the aisles in their barn (approximately 100 m for 10 min). Pigs were exercised to simulate the stresses on the muscles experienced during slaughter.

Forty-five minutes after oral treatment, pigs were snared and bled via the anterior vena cava into a heparinized vacuum tube (BD Vacutainer, Franklin Lakes, NJ). The tubes were stored on ice for no more than 4 h and were taken to the Clinical Pathology Laboratory at the Kansas State University Veterinary Diagnostic Laboratory. Blood was analyzed for pH and lactate by using a Stat Profile M Analyzer (Nova Biomedical, Waltham, MA). Bicarbonate levels were also calculated. The pigs were allowed to rest in their pens for another 30 min and were bled again.

Exp. 2

Two replications (one of 15 gilts and one of 5 gilts and 10 barrows) were fed a standard finishing diet containing 20 mg/kg of ractopamine for 14 d before slaughter. Pigs were assigned by BW and sex to 10 groups of 3, and pigs in each BW group were assigned to the CIT, ACE, or control antemortem treatment. Before slaughter, the pigs were fasted for no less than 20 h before transportation to the Kansas State University Meat Laboratory. Final BW ranged from 104 to 150 kg. At 45 min before slaughter, the pigs were restrained with a snout snare and administered their antemortem treatments, as described in Exp. 1. Pigs were slaughtered in random order within their BW group.

Slaughter. Immediately before slaughter, pigs were bled, and blood was analyzed for pH and lactate, as described in Exp. 1. After the blood samples were collected, the pigs were gently moved to the abattoir and stunned with an electric stunning wand and stunned again with a captive bolt stunner to ensure complete unconsciousness. Pigs continued through the standard slaughter procedures of exsanguination, scalding, evisceration, and trim. After a final inspection and wash, the carcasses were tagged for identification, and a temperature logger (Hobo XT, Onset Computer Corporation, Bourne, MA) was placed in the inside ham (semimembranosus muscle, **SM**). The probe was positioned at a 45° angle to the skin surface so that the tip of the probe was 15 cm from the inside of the ham surface. The data loggers read the temperature every 2.4 min for 24 h. Carcasses were chilled to 1°C at approximately 45 min postmortem.

At 20 min poststunning, a sample (approximately 2.54-cm-thick chop) was removed from the anterior portion of the LM (immediately posterior to the scapula), cut into cubes, and frozen in liquid nitrogen. Additional samples were removed at 45 min and at 3, 6, 12, and 24 h postmortem. Samples removed at 45 min, 3 h, and 12 h were used to determine glucose-6 phosphate (**G6P**), F6P, F16BP, the combination of glyceraldehyde-3 phosphate and dihydroxyacetone phosphate (**GAP-DAP**), and lactate. Early postmortem pH was analyzed on 20-min, 45-min, 3-h, 6-h, 12-h, and 24-h muscle samples. Cubes were packaged in whirl-pack bags, stored on dry ice for no more than 6 h, and stored at –80°C until analysis. Samples were removed from the LM of

the same side at 45 min, and at 3, 6, 12, and 24 h postmortem and stored at –80°C. All LM samples were removed at least 2.54 cm apart to ensure that the pH and glycolytic metabolite data were not affected by the chilling rate of the previously cut surfaces.

Early Postmortem pH. Cubes of muscle frozen in liquid nitrogen were stored at –80°C until they were removed from their bags and pulverized in a Waring blender for pH and glycolytic metabolite analyses. Duplicate 5-g samples of pulverized muscle were weighed into 100-mL beakers and were mixed in a 1:2 (wt/vol) solution of 2.0 *M* iodoacetate in 150 m*M* potassium chloride to arrest glycolysis. Beakers were covered with Parafilm (American National Can, Menasha, WI) and allowed to acclimate to room temperature on the benchtop for no less than 4 h. The solution was mixed again, and the pH of the duplicate samples was measured with an Accumet Portable pH meter and probe (model AP61, Fisher Scientific, Fairlawn, NJ).

Glycolytic Metabolite Analysis. The water-soluble components of the LM were extracted according to the extraction procedure described by Bergmeyer (1974), and G6P, F6P, F16BP, GAPDAP, and lactate were determined in the extract by using spectrophotometric methods, as described below. Approximately 1 g of frozen, pulverized muscle tissue was added to a 15-mL centrifuge tube containing 5 mL of perchloric acid (1 *N*); the tube was capped and vortexed. Tubes were centrifuged with a Beckman centrifuge (model J2-21, Beckman Coulter Inc., Fullerton, CA) at 3,000 × *g* for 10 min, and the supernatant was transferred to a second 15-mL centrifuge tube. One milliliter of perchloric acid and 1 mL of water were added to the pellet, centrifugation was repeated, and the supernatants were combined. Methyl orange indicator (0.02 mL) was added to the solution, and the pH was brought to 3.5; a 5 *M* solution of potassium carbonate was used to make large adjustments in pH, and a 0.5 *M* solution was used to make fine adjustments. The final volume of the tube was brought to the nearest milliliter with distilled, deionized water and the volume was recorded. Samples were held in an ice bath for no less than 10 min, and were stored at 1°C for no more than 4 wk until analysis.

Determination of G6P and F6P was carried out according to procedures described by Bergmeyer (1974), with minor modification. Buffer (pH 7.6) was prepared with 18.6 g of triethanolamine hydrochloride and 18 mL of 2 *N* sodium hydroxide, and was brought to 250 mL with distilled, deionized water. Cuvettes were prepared with 1.9 mL of buffer, 0.1 mL of sample extract, 0.01 mL of 20 m*M* nicotinamide adenine dinucleotide phosphate (NADP), and 0.02 mL of magnesium chloride (0.5 *M*). Cuvettes were mixed, and absorbance was read twice at 5-min intervals at 340 nm on a spectrophotometer (model U-2010, Hitachi High Technologies America Inc., San Jose, CA). Glucose-6 phosphate dehydrogenase enzyme (0.737 U/cuvette, Sigma-Aldrich, St. Louis, MO) was added to the cuvettes, mixed, and allowed to react for 5 to 8 min. After the reaction occurred, absorbance was measured twice at 5-min intervals to determine G6P concentrations. Similar absorbances of the readings that were 5 min apart indicated that the reaction had reached completion. Phosphoglucose isomerase (2.07 U/cuvette, Sigma-Aldrich) was added to the cuvette, mixed, and allowed to react for 5 to 8 min. Two absorbance readings were made at 5-min intervals to determine F6P concentrations. Increasing absorbance indicated production of nicotinamide adenine dinucleotide phosphate (NADPH) from oxidized NADP. One molecule of NADPH was formed for each G6P or F6P molecule present (Bergmeyer, 1974).

Determination of F16BP and GAPDAP was completed according to the methods of Bergmeyer (1974). Buffer (pH 7.6) was prepared with 18.6 g of triethanolamine, 3.7 g of EDTA, and 18 mL of sodium hydroxide, and was brought to 250 mL with distilled, deionized water. Cuvettes were prepared with 1.5 mL of buffer, 1.5 mL of sample extract, and 0.02 mL of 20 m*M* NADH. Two absorbance readings (340 nm) were taken at 5-min intervals before the addition of glycerophosphate dehydrogenase (2.07 U/cuvette, Sigma-Aldrich) and triosephosphate isomerase (2.50 U/cuvette, Sigma-Aldrich) enzymes. The reaction was allowed to occur for 15 min, and absorbance was read twice at 5-min intervals for determination of GAP and DAP. Concentrations of DAP are generally quite low in muscle (Bergmeyer, 1974), so these 2 metabolites were measured in concert. Aldolase enzyme (1.95 U/cuvette, Sigma-Aldrich) was added to the cuvettes, mixed, and allowed to react for 9 min. Two absorbance readings were taken at 5-min intervals to determine F16BP. Decreasing absorbance indicated a loss of NADH to form NAD. Two NADH molecules are used for each F16BP present, and one NADH molecule is used for each GAP or DAP present (Bergmeyer, 1974).

Lactate concentration was determined according to the procedure of S. B. Smith (2005, Texas A&M University, personal communication). Buffer was prepared with 3.8 g of glycine, 4.0 mL of 85% hydrazine hydrate, 76 mg of EDTA, 8 mL of 1 *N* sodium hydroxide, and distilled, deionized water to make approximately 90 mL of solution. The pH was adjusted to 9.4 with sodium hydroxide, and the final volume was brought to 100 mL by adding distilled water. Cuvettes were prepared with 1.95 mL of buffer, 2 mg of NAD in 0.2 mL of water, 0.9 mL of water, and 0.05 mL of sample extract. Absorbance was read at 340 nm, lactic dehydrogenase (1.55 U/cuvette, Sigma-Aldrich) was added, and the reaction occurred for 90 min. A second absorbance reading was made to determine the lactate concentrations. Absorbance increased with increasing NADH production, and one NADH was produced for each lactate molecule present.

Fabrication and Visual Evaluation. At 24 h postmortem, carcasses were fabricated into wholesale loins (IMPS 410) and hams (IMPS 401A), which were further processed into chops or slices for pork quality data collection. Two 2.54-cm-thick LM chops were removed

from the loin, and one chop was allowed to bloom for no less than 30 min before visual evaluation. Three trained panelists evaluated each chop for color, firmness, and wetness. Color was evaluated on a 6-point scale, according to the official color standards of the National Pork Producers Council (1999; 1 = lightest to 6 = darkest), whereas firmness and wetness were evaluated separately using 3-point scales (1 = softest and wettest to 3 = firmest and driest). Chops were evaluated in random order within BW groups. The SM (inside ham) was excised from the ham, and 2.54-cm-thick slices were allowed to bloom for no less than 30 min before visual evaluation. Inside and outside sections of the SM slices were evaluated separately for color, firmness, and wetness, as described for LM chops.

After visual evaluation, a Miniscan XE Plus spectrophotometer (1.4-cm aperture, Illuminant D_{65}, and 10° observer; Model D/8-S, Hunter Associates Laboratory, Reston, VA) was used to evaluate L*, a*, and b* on the chops and ham slices. Four measurements were made on each LM chop and were averaged for analysis, whereas 2 measurements were made on each of the inside and outside sections of the SM slices and were averaged for analysis.

The second LM chop was vacuum-packaged for the measurement of expressible moisture and ultimate pH. The inside and outside sections of the SM slices were separated with a knife and vacuum-packaged. Chops and steaks were held at 1°C for expressible moisture at 2 d postmortem and for ultimate pH at 3 d postmortem.

Expressible Moisture. At 2 d postmortem, chops and slices were removed from their packages, and duplicate 2- to 3-g samples were cut from the interior of the muscle. Samples were weighed and placed in a 50-mL centrifuge tube fitted with one piece of 11-cm-diameter Whatman no. 3 filter paper folded around one piece of 5-cm-diameter Whatman no. 50 filter paper (Whatman International Ltd., Maidstone, UK). Tubes were capped and centrifuged at $2{,}400 \times g$ for 10 min with a Beckman centrifuge (model J2-21, Beckman Coulter Inc.). After centrifugation, samples were weighed again to calculate expressible moisture {[(in weight – out weight)/in weight)] × 100}. The duplicate samples were averaged for analysis. After the expressible moisture samples were removed, the remainder of the sample was revacuum-packaged and held at 1°C for pH analysis.

Ultimate pH. At 3 d postmortem, duplicate 10-g samples of LM and SM were minced with a scalpel, placed in filtered stomacher bags with 100 mL of distilled water, and mechanically agitated for 2 min in a Stomacher 400 (Seward Ltd., Worthing, UK). After samples were stomached, an Accumet pH probe, attached to a portable Accumet meter, was placed in the solution in the bag, and the pH was read for each duplicate sample. Readings for duplicates were averaged for statistical analysis.

Statistical Analysis

Blood parameters were analyzed in a randomized, complete block design, with BW group as the block. Temperature measurements were analyzed in a randomized block design, with repeated measures and BW group as the block. Early postmortem pH and glycolytic metabolite data were analyzed in a split-plot design, with antemortem treatment as the whole plot and time postmortem as the subplot. The whole plot was blocked by BW group. Instrumental color, expressible moisture, and ultimate pH were analyzed in a randomized, complete block design with BW group as the block. Antemortem treatment and time postmortem were the fixed effects, and BW group was the random effect. Visual color, firmness, and wetness were analyzed in a randomized, complete block design with panelist as the block. Antemortem treatment was again the fixed effect and panelist was the random effect. Data were analyzed by using PROC MIXED (SAS Inst. Inc., Cary, NC), and least squares means were separated by using the PDIFF test when $P < 0.05$. For repeated measures analysis, the repeated measures command was used with the autoregressive option.

Table 1. Effect of oral administration of sodium acetate (ACE) or sodium citrate (CIT) on blood pH, bicarbonate, and lactate concentrations 45 and 75 min after treatment in Exp. 1 (n = 8/treatment)

Item	ACE	CIT	Control	SE[1]
45 min[2]				
pH	7.35[x]	7.36[x]	7.31[y]	0.02
Bicarbonate, mmol/L	29.85[a]	27.03[a]	26.30[b]	1.17
Lactate, mmol/L	9.53	10.75	9.98	1.18
75 min				
pH	7.46[x]	7.41[y]	7.41[y]	0.02
Bicarbonate, mmol/L	33.99[a]	31.05[b]	28.38[c]	1.11
Lactate, mmol/L	6.19	7.34	8.39	1.18

[a–c]Within a row, least squares means lacking a common superscript letter differ ($P < 0.05$).
[x,y]Within a row, least squares means lacking a common superscript letter differ ($P < 0.10$).
[1]Largest SE for antemortem treatments.
[2]At 30 min after treatment, the pigs were exercised before being bled.

RESULTS AND DISCUSSION

Exp. 1

After pigs were exercised, those administered CIT and ACE tended ($P = 0.08$) to have greater blood pH and had higher ($P = 0.02$) bicarbonate levels (45 min after treatment; Table 1). Linossier et al. (1997) and Kowalchuk et al. (1989) found that exercise increased hydrogen ion concentration in exercising humans and that the increase was not as severe when CIT was administered before the exercise bout. It is likely that the exercise bout of the pigs in our study decreased blood pH (increased hydrogen ion concentration) and that the CIT and ACE helped to buffer the decrease. After pigs were allowed to rest for 30 min, the blood pH of pigs administered CIT had returned to that of the control

Table 2. Effect of oral administration of sodium acetate (ACE) or sodium citrate (CIT) on blood pH, bicarbonate concentration, and lactate concentration 45 min after treatment in Exp. 2 (n = 10/treatment)

Item	ACE	CIT	Control	SE[1]
pH	7.48	7.47	7.48	0.01
Bicarbonate, mmol/L	37.36	37.08	37.31	0.65
Lactate, mmol/L	2.51[ab]	3.36[a]	1.51[b]	0.45

[a,b]Within a row, least squares means lacking common superscript letters differ ($P < 0.05$).
[1]Largest SE for antemortem treatments.

pigs ($P = 0.92$), whereas those pigs administered ACE still tended to have higher blood pH ($P = 0.09$) than control pigs. Although blood lactate levels were similar ($P = 0.26$) at 75 min, bicarbonate levels from ACE- and CIT-treated pigs remained higher ($P \leq 0.05$) than those of control pigs. These results were promising for the effects of CIT and ACE on blood pH and potentially early postmortem muscle metabolism.

Exp. 2

Blood Parameters. Mean values for blood pH, bicarbonate concentration, and lactate concentration at 45 min after oral administration of the CIT, ACE, or control treatment are presented in Table 2. Ingestion of CIT or ACE did not affect blood pH or bicarbonate levels. Lactate concentration in pigs orally administered CIT was greater ($P < 0.05$) than that in controls, but lactate levels of pigs treated with ACE did not differ ($P > 0.05$) from those of control pigs or pigs treated with CIT. In Exp. 1, both CIT and ACE treatments showed an alkalizing effect on blood after exercise, whereas in Exp. 2, the pigs were not exercised before blood sampling. As stated earlier, the exercise likely lowered blood pH in Exp. 1; however, in Exp. 2, no exercise bout was experienced to lower blood pH, and the CIT and ACE treatments did not have the opportunity to buffer the pH decline. Citrate is an alkaline substance that has been shown to increase blood concentrations of CIT (Kowalchuk et al., 1989; Linossier et al., 1997) as well as increase blood pH, bicarbonate, and lactate (Kowalchuk et al., 1989; Potteiger et al., 1996a,b; Linossier et al., 1997) in exercising humans. Increased lactate concentrations were attributed to increased blood pH and the flux of lactate out of working muscles into the blood to maintain blood pH (Potteiger et al., 1996b). Pigs treated with CIT had elevated lactate concentrations, but pH was not affected by CIT or ACE in Exp. 2, in which pigs were not exercised. Greater lactate levels were likely a function of the animals' maintaining homeostasis through the flux of lactate and hydrogen ions out of the muscle to regulate blood pH in response to the antemortem treatments.

Postmortem Glycolysis and Early Postmortem pH. Antemortem treatment did not affect ($P > 0.05$) concentrations of glycolytic metabolites during postmortem glycolysis (Table 3) or pH at 20 min, 45 min, or 3, 6, 12, or 24 h ($P > 0.05$; Table 4). Even though we did not measure myocellular CIT concentrations in our study, it has been reported in human studies in which glycolytic rate was increased via exercise that CIT supplementation (0.5 g/kg of BW) increased intracellular CIT (Linossier et al., 1997) and that feeding ACE increases i.m. CIT concentrations in mice (Fushimi et al., 2001). Ingestion of CIT before exercise has been shown to increase blood pH and improve performance in human athletes (Potteiger et al., 1996a,b; Linossier et al., 1997), but it has also been shown not to have an effect on exercise performance (Kowalchuk et al., 1989). Exercise physiologists theorize that athletes with more alkaline conditions in their blood during exercise have enhanced performance over athletes with neutral and acidotic blood conditions (Kowalchuk et al., 1989; Potteiger et al., 1996a,b; Linossier et al., 1997). The extreme effects of slaughter on the body (i.e., accelerated anaerobic metabolism, decrease in muscle pH, loss of blood and oxygen supply to the muscle, and loss of homeostasis) were assumed to be somewhat similar to exhaustive anaerobic exercise, but neither CIT nor ACE administration affected postmortem muscle pH conditions in our study. Injection of CIT directly into prerigor beef inhibited postmortem glycolysis and improved tenderness (Jerez et al., 2003). However, injecting CIT into prerigor porcine muscle elevated pH, but neither CIT nor ACE administration had an effect on glycolytic metabolites (Stephens et al., 2006).

Aside from increasing blood pH, CIT administration affects metabolism by inhibiting PFK in muscle (Newsholme et al., 1977), thus having the potential to inhibit glycolysis and postmortem pH decline. Acetate ingestion increased postmortem muscle CIT concentrations and decreased PFK activity in rats (Fushimi et al., 2001). These researchers concluded that ACE was converted to CIT in the body by citrate synthase and that CIT inhibited glycolysis as described previously. Nonetheless, neither CIT nor ACE administration altered glycolytic metabolite levels in our study.

Samples taken at 20 min postmortem had the highest pH ($P < 0.05$), and mean values of pH were higher ($P < 0.05$) at 45 min than the remaining times postmortem. However, pH values at 3, 6, 12, and 24 h postmortem did not differ ($P > 0.05$). Temperature declined over time and did not differ ($P > 0.05$) among antemortem treatments (results not shown).

PFK over Time. No interactions between treatments and time postmortem were found for the glycolytic metabolite results presented in Table 3. Concentrations of G6P and F6P increased ($P < 0.05$) as postmortem time increased, whereas F16BP and GAPDAP decreased ($P < 0.05$) with postmortem time. Lactate is the final product of glycolysis, and lactate accumulated ($P < 0.05$) with time (Table 3). Dalrymple and Hamm (1975) and Rhoades et al. (2005) stated that PFK is a major role player in regulation of postmortem glycolysis, because metabolites that serve as substrates for PFK accumu-

Table 3. Effect of oral administration of sodium acetate (ACE) or sodium citrate (CIT) 45 min before slaughter on glycolytic metabolites in LM samples removed 45 min, 3, and 12 h postmortem (n = 10/antemortem treatment)

	Antemortem treatment			Time postmortem		
LM metabolite[1]	ACE	CIT	Control	45 min	3 h	12 h
G6P, μmol/g	6.59	6.93	6.27	4.73[c]	7.15[b]	7.91[a]
SE	0.42	0.42	0.42	0.29	0.29	0.29
F6P, μmol/g	0.89	0.84	0.83	0.73[b]	0.94[a]	0.89[a]
SE	0.05	0.05	0.05	0.04	0.04	0.04
F16BP, nmol/g	43.0	42.5	35.9	87.5[a]	17.6[b]	16.4[b]
SE	5.6	5.7	5.7	5.3	5.2	5.2
GAPDAP, nmol/g	21.9	25.4	16.4	39.5[a]	9.6[b]	14.5[b]
SE	3.4	3.4	3.4	3.0	3.0	3.0
Lactate, μmol/g	10.83	10.76	11.18	9.29[b]	11.90[a]	11.58[a]
SE	0.34	0.34	0.34	0.34	0.34	0.34

[a–c]Within a row and main effect, least squares means lacking common superscript letters differ ($P < 0.05$).
[1]G6P = glucose-6 phosphate; F6P = fructose-6 phosphate; F16BP = fructose-1,6 bisphosphate; GAPDAP = glyceraldehyde-3 phosphate and dihydroxyacetone phosphate.

late in postmortem muscle, and PFK products (with the exception of lactate) decrease with time. Glycolytic metabolite results are in agreement with those of Dalrymple and Hamm (1975) and Rhoades et al. (2005), who noted that G6P and F6P increased with postmortem time, whereas F16BP and GAPDAP decreased.

Ratios of glycolytic metabolites at 3 h and 45 min postmortem, 12 h and 3 h postmortem, and 12 h and 45 min postmortem were calculated for each metabolite (Figure 1). This figure was derived from the mean values of each treatment at the respective times postmortem (Rhoades et al., 2005). The metabolites are presented from left to right in the order they occurred in the glycolysis reaction. Ratios greater than 100 indicate that the metabolite increased with time, and ratios less than 100 indicate that the metabolite decreased with time. When the line between 2 metabolites crossed from greater than 100 to less than 100, the enzyme between them was considered rate limiting (Rhoades et al., 2005).

Ratios of 3-h to 45-min values for G6P and F6P were greater than 100, whereas those for F16BP and GAPDAP were less than 100 (Figure 1). This pattern is similar to that reported by Rhoades et al. (2005), and indicated that the rate-limiting step of glycolysis occurred between F6P and F16BP, or at PFK. The pre-PFK metabolites accumulated, whereas the post-PFK metabolites were used over time without replenishment. Values for G6P, F6P, and lactate were close to 100, whereas ratios for F16BP and GAPDAP were greater than 100 (Figure 1). These patterns indicated that there was no rate-limiting step for the reaction between the times of 3 and 12 h postmortem. The patterns of the ratios of 12-h to 45-min values were similar to those of 3 h to 45 min (Figure 1). This was not surprising because the values for 3 h and 12 h were similar ($P > 0.05$) for the glycolytic metabolites measured (except G6P). The relationships of the glycolytic metabolite values at the different times postmortem indicated that PFK limited glycolysis between 45 min and 3 h postmortem but that the effects of PFK on glycolysis were minimal after 3 h postmortem.

Pork Quality Traits. Visual color scores for the LM chops from ACE-treated pigs were greater (indicating a darker color; $P < 0.05$) than those from control pigs, whereas LM chops from CIT-treated pigs were intermediate (Table 5). Loin chops from pigs treated with ACE also had greater ($P < 0.05$) scores for wetness (drier) than those from CIT-treated pigs and control pigs; however, firmness scores did not differ ($P > 0.05$) among

Table 4. Effect of oral administration of sodium acetate (ACE) or sodium citrate (CIT) 45 min before slaughter on postmortem pH decline in LM (n = 10/antemortem treatment)

	Time postmortem							
Item	20 min	45 min	3 h	6 h	12 h	24 h	Mean	SE
ACE	6.17	5.92	5.53	5.50	5.50	5.46	5.68	0.04
CIT	6.21	5.94	5.47	5.44	5.45	5.39	5.65	0.04
Control	6.20	5.95	5.39	5.41	5.44	5.44	5.64	0.04
Mean	6.19[a]	5.94[b]	5.46[c]	5.45[c]	5.46[c]	5.43[c]		
SE	0.03	0.03	0.03	0.03	0.03	0.03		

[a–c]Within a row, least squares means lacking common superscript letters differ ($P < 0.05$).

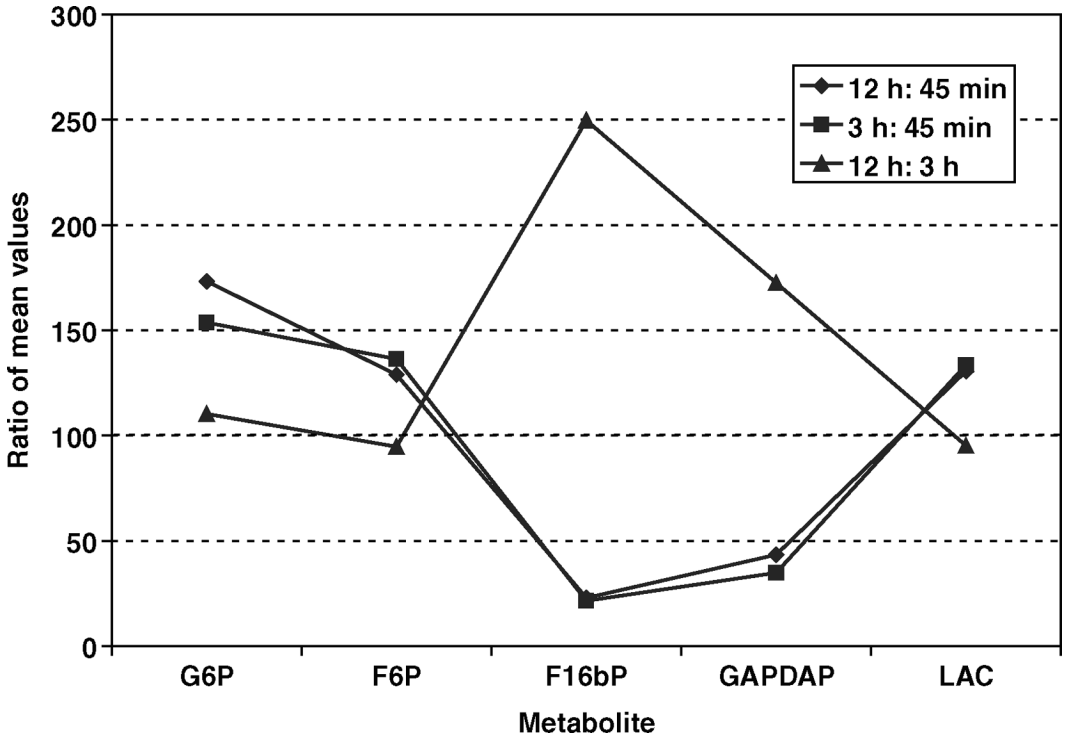

Figure 1. Crossover diagram for glycolytic metabolites of pork LM from pigs orally administered sodium citrate, sodium acetate, or water as control. Data points are a ratio of (mean at later time/mean at earlier time) × 100. Each point is a mean of the 3 treatments. Ratios greater than 100 indicate that the metabolite increased with time, and ratios less than 100 indicate that the metabolite decreased with time. When the line between 2 metabolites crosses from greater than 100 to less than 100, the enzyme between them is considered rate limiting. G6P = glucose-6 phosphate ($\mu mol \cdot g^{-1}$); F6P = fructose-6 phosphate ($\mu mol \cdot g^{-1}$); F16BP = fructose-1,6 bisphosphate ($nmol \cdot g^{-1}$); GAPDAP = glyceraldehyde-3 phosphate and dihydroxyacetone phosphate ($nmol \cdot g^{-1}$); LAC = lactate ($\mu mol \cdot g^{-1}$).

antemortem treatments. Chops from CIT-treated pigs were lighter (greater L* value; $P < 0.05$) and more yellow (greater b* value, $P < 0.05$) than chops from control or ACE-treated pigs. Conversely, LM chops from CIT-treated pigs also had greater ($P < 0.05$) a* values (more red) than chops from control pigs, whereas a* values for LM chops from ACE-treated pigs were intermediate. Neither expressible moisture, nor ultimate pH values of the LM, nor any pork quality attribute of the inside and outside SM differed ($P > 0.05$) among antemortem treatments. Thus, neither CIT nor ACE was effective at inhibiting postmortem glycolysis or slowing pH decline and, as a consequence, had no beneficial effects on pork quality.

There are several possibilities why CIT and ACE failed to alter muscle metabolism and pork quality in the second experiment. First, in other studies, CIT was administered 90 min before exercise (Potteiger et al., 1996a,b; Linossier et al., 1997), and ACE was fed 2 h before slaughter (Fushimi et al., 2001). However, pigs in our study were orally administered a larger dose of CIT and ACE than used in human experiments (0.75 g/kg vs. 0.3 and 0.5 g/kg) 45 min before slaughter. It was hypothesized that the larger dose would induce a peak in blood pH in less time than would a smaller dose. In Exp. 1, 0.75 g/kg of BW of CIT and ACE appeared to have maximal effects on blood pH of pigs when administered 45 min before exercise and blood sampling. Moreover, James et al. (2004) found that, by 1 h after extremely stressful handling procedures, blood pH, lactate, bicarbonate, and other metabolites were returned to prehandling levels.

In contrast to Exp. 1, pigs in Exp. 2 were not exercised before blood sampling, and CIT and ACE could not buffer the subsequent decline in blood pH seen in Exp 1. Perhaps results from Exp. 1 were misinterpreted, and altering the peak of blood pH should not have been the goal. If CIT was having maximal effects on the blood, it may not have had time to cross the membrane into the muscle. Furthermore, exsanguination may have removed most of the CIT and ACE in the blood before the compounds had time to affect muscle metabolism; thus, more time between CIT or ACE administration and slaughter may have altered the response from Exp. 1 to Exp. 2.

The stressful handling during bleeding and strange surroundings associated with the preslaughter protocol were likely to have stimulated glycolysis prematurely (van Laack, 2000). Moreover, pigs were stunned with a captive-bolt stunner, which induced kicking during exsanguination. This extra activity may have caused the pigs to metabolize glucose stores while the blood

Table 5. Effect of oral administration of sodium acetate (ACE) or sodium citrate (CIT) 45 min before slaughter on pork quality characteristics of the LM and semimembranosus muscle (SM; n = 10/treatment)

		Treatment			
Muscle	Item	ACE	CIT	Control	SEM
LM	Color[1]	2.91[a]	2.73[ab]	2.55[b]	0.08
	Firmness[2]	2.28	2.13	2.15	0.11
	Wetness[2]	2.38[a]	2.08[b]	2.11[b]	0.11
	L*[3]	56.41[b]	60.34[a]	57.68[b]	0.84
	a*[4]	3.36[ab]	4.11[a]	2.57[b]	0.43
	b*[5]	12.36[b]	13.53[a]	12.18[b]	0.44
	Expressible moisture	21.34	23.75	23.34	1.03
	Ultimate pH	5.53	5.38	5.53	0.05
Inside SM	Color	2.59	2.53	2.73	0.15
	Firmness	1.98	2.00	2.02	0.09
	Wetness	2.03	2.05	2.08	0.13
	L*	57.14	56.82	58.24	1.09
	a*	7.05	7.36	6.96	0.61
	b*	15.48	15.70	15.50	0.50
	Expressible moisture	22.48	20.13	19.81	0.89
	Ultimate pH	5.59	5.58	5.60	0.04
Outside SM	Color	2.98	2.91	3.08	0.38
	Firmness	2.26	2.22	2.32	0.11
	Wetness	2.30	2.17	2.27	0.15
	L*	55.75	55.97	56.07	0.93
	a*	5.20	5.17	4.73	0.53
	b*	14.32	14.64	14.21	0.51
	Expressible moisture	20.98	21.66	20.48	1.18
	Ultimate pH	5.64	5.60	5.62	0.03

[a,b]Within a row, least squares means lacking common superscript letters differ ($P < 0.05$).

[1]Color was evaluated on a 6-point scale using the official color standards from the National Pork Producers Council (1 = lightest and 6 = darkest).

[2]Firmness and wetness were evaluated separately on 3-point scales (1 = softest and wettest and 3 = firmest and driest).

[3]L* is a measure of lightness (greater number is a lighter color).

[4]a* is a measure of redness (greater number is a redder color).

[5]b* is a measure of yellowness (greater number is a more yellow color).

system was still intact to remove excess lactate and buffer pH changes. In addition, the effects of slaughter on the body may be so extreme that any effect that glycolytic inhibitors may have on a living system are overwhelmed by the severity of loss of homeostasis, loss of blood, and rigor onset.

Kemp and Krebs (1967) reported that low concentrations of CIT and nonsaturating concentrations of ATP increased the affinity of the PFK substrate site for ATP, and PFK activity was increased. Rigor onset is, by definition, a decrease in ATP in the muscle, resulting in a nonsaturated condition (Hamm, 1977). Therefore, low concentrations of ATP, in combination with low concentrations of CIT in the cytosol caused by loss during exsanguination, may have served as an activator for PFK, thereby improving its affinity for ATP at the substrate site rather than serving as an inhibitor in postmortem muscle. Interestingly, Allison et al. (2003) found that glycolytic enzyme activity was not related to pork quality attributes, such as water-holding capacity and pale color, and they speculated that PFK was denatured by 20 min postmortem because of low pH. If their conclusion is valid, any inhibition that CIT or ACE may have on PFK would not have had an effect on pork quality attributes, explaining the lack of differences among our treatments.

Conclusions

Oral administration of CIT and ACE was successful in increasing blood pH and bicarbonate levels in exercised pigs; however, neither CIT nor ACE administration before slaughter effectively inhibited postmortem glycolysis, altered early postmortem pH, or improved pork quality. The novelty of this study was the establishment of reference doses for future CIT and ACE supplementation studies. Phosphofructokinase was the rate-limiting step in postmortem glycolysis, and evidence suggests that glycolytic inhibitors may have potential to affect postmortem metabolism in pork, but these inhibitors must reach the enzyme in time to be effective early postmortem.

LITERATURE CITED

Allison, C. P., R. O. Bates, A. M. Booren, R. C. Johnson, and M. E. Doumit. 2003. Pork quality variation is not explained by glycolytic enzyme capacity. Meat Sci. 63:17–22.

Bergmeyer, H. U. 1974. Pages 1125–1624 in Methods of Enzymatic Analysis. Vol. 3. Academic Press, New York, NY.

Dalrymple, R. H., and R. Hamm. 1975. Postmortem glycolysis in prerigor ground bovine and rabbit muscle. J. Food Sci. 40:850–853.

Fushimi, T., K. Tayama, M. Fukaya, K. Kitakohi, N. Nakai, Y. Tsukamoto, and Y. Sato. 2001. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. J. Nutr. 131:1973–1977.

Hamm, R. 1977. Postmortem breakdown of ATP and glycogen in ground muscle: A review. Meat Sci. 1:15–39.

James, B. W., M. D. Tokach, R. D. Goodband, J. L. Nelssen, S. S. Dritz, J. M. DeRouchey, and J. C. Woodworth. 2004. Effect of dietary L-carnitine and ractopamine-HCL (Paylean) on the metabolic response to handling in growing-finishing pigs. Pages 158–171 Swine Day 2004. R. D. Goodband, M. Tokach, S. Dritz, and J. DeRouchey, ed. Kansas State University, Manhattan.

Jerez, N. C., C. R. Calkins, and J. Velazco. 2003. Prerigor injection using glycolytic inhibitors in low-quality beef muscles. J. Anim. Sci. 81:997–1003.

Kemp, R. G., and E. G. Krebs. 1967. Binding of metabolites by phosphofructokinase. Biochemistry 6:423–434.

Kowalchuk, J. M., S. A. Maltais, K. Yamaji, and R. L. Hughson. 1989. The effect of citrate loading on exercise performance, acid-base balance, and metabolism. Eur. J. Appl. Physiol. 58:858–864.

Linossier, M. T., D. Dormois, P. Brégère, A. Geyssant, and C. Denis. 1997. Effect of sodium citrate on performance and metabolism of human skeletal muscle during supramaximal cycling exercise Eur. J. Appl. Phys. 76:48–54.

Newsholme, E. A., P. H. Sugden, and T. Williams. 1977. Effects of citrate on the activities of 6-phosphofructokinase from nervous and muscle tissues from different animals and its relationship to the regulation of glycolysis. Biochem. J. 166:123–129.

National Pork Producers Council. 1999. Pork Quality Standards. Natl. Pork Producers Counc., Des Moines, IA.

Potteiger, J. A., G. L. Nickel, M. J. Webster, M. D. Haub, and R. J. Palmer. 1996a. Sodium citrate ingestion enhances 30 km cycling performance. Int. J. Sports Med. 17:7–11.

Potteiger, J. A., M. J. Webster, G. L. Nickel, M. D. Haub, and R. J. Palmer. 1996b. The effects of buffer ingestion on metabolic factors related to distance running performance. Eur. J. Appl. Physiol. 72:365–371.

Puolanne, E. J., A. R. Ps, M. H. Ruusunen, K. V. Sepponen, and M. S. Kylä-Puhju. 2002. Lactic acid in muscle and its effects on meat quality. Pages 57–62 in Proc. 55th Recip. Meat Conf., East Lansing, MI. Am. Meat Sci. Assoc., Savoy, IL.

Rhoades, R. D., D. A. King, B. E. Jenschke, J. M. Behrends, T. S. Hively, and S. B. Smith. 2005. Postmortem regulation of glycolysis by 6-phosphofructokinase in bovine M. sternocephalicus pars mandibularis. Meat Sci. 70:621–626.

Sayre, R. N., and E. J. Briskey. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28:675–679.

Stephens, J. W., M. E. Dikeman, J. A. Unruh, M. D. Haub, and M. D. Tokach. 2006. Effects of pre-rigor injection of sodium citrate or acetate, or post rigor injection of phosphate plus salt on postmortem glycolysis, pH, and pork quality attributes. Meat Sci. 74:727–737.

van Laack, R. L. J. M. 2000. Determination of ultimate pH of meat and poultry. Pages 74–75 in Proc. 53rd Recip. Meat Conf., Columbus, OH. Am. Meat Sci. Assoc., Savoy, IL.