Effects of dietary L-carnitine and ractopamine HCl on the metabolic response to handling in finishing pigs[1,2]

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ABSTRACT: Two experiments (384 pigs; C22 × L326; PIC) were conducted to determine the interactive effect of dietary L-carnitine and ractopamine HCl (RAC) on the metabolic response of pigs to handling. Experiments were arranged as split-split plots with handling as the main plot and diets as subplots (4 pens per treatment). Dietary L-carnitine (0 or 50 mg/kg) was fed from 36.0 kg to the end of the experiments (118 kg), and RAC (0 or 20 mg/kg) was fed the last 4 wk of each experiment. At the end of each experiment, 4 pigs per pen were assigned to 1 of 2 handling treatments. Gently handled pigs were moved at a moderate walking pace 3 times through a 50-m course and up and down a 15° loading ramp. Aggressively handled pigs were moved as fast as possible 3 times through the same course, but up and down a 30° ramp, and shocked 3 times with 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, but there was no effect ($P > 0.10$) of L-carnitine on growth performance. In Exp. 1 and 2, aggressive handling increased ($P < 0.01$) blood lactate dehydrogenase (LDH), lactate, cortisol, and rectal temperature and decreased blood pH. In Exp. 1, there was a RAC × handling interaction ($P < 0.06$) for the difference in pre- and posthandling blood pH and rectal temperature. Aggressively handled pigs fed RAC had decreased blood pH and increased rectal temperature compared with gently handled pigs, demonstrating the validity of the handling model. Pigs fed RAC had increased ($P < 0.01$) LDH compared with pigs not fed RAC. Pigs fed L-carnitine had increased ($P < 0.03$) lactate compared with pigs not fed L-carnitine. In Exp. 2, pigs fed RAC had lower ($P < 0.02$) blood pH immediately after handling, but pH returned to control levels by 1 h posthandling. Lactate, LDH, cortisol, and rectal temperature changes from immediately posthandling to 1 h posthandling were not different ($P > 0.10$) between pigs fed L-carnitine and those fed RAC, indicating that L-carnitine did not decrease recovery time of pigs subjected to aggressive handling. These results suggest that pigs fed 20 mg/kg of RAC are more susceptible to stress when handled aggressively compared with pigs not fed RAC. Dietary L-carnitine fed in combination with RAC did not alleviate the effects of stress. This research emphasizes the importance of using proper animal handling techniques when marketing finishing pigs fed RAC.

Key words: animal handling, L-carnitine, pigs, ractopamine HCl

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INTRODUCTION

Downer pigs are those that become fatigued, refuse to get up and walk, or cannot keep up with other pigs during loading, unloading, or moving through the packing plant (Grandin, 1998). Approximately 0.1 to 0.5% of pigs are characterized as downer pigs in commercial pork processing plants (Carr et al., 2005). Pigs that die or become nonambulatory during transportation or at the packing

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plant cost the United States swine industry about US$100 million annually (Ellis et al., 2003). The prevalence of downer pigs has been attributed to several factors including handling, genetics, and muscling. Aggressive handling of pigs results in increased concentrations of serum lactate, decreased blood pH, and increased incidence of downer pigs (Anderson et al., 2002) and may occur more frequently because of the industry trend of producing lean, heavily muscled pigs (Grandin, 1998).

Ractopamine HCl (**RAC**; Paylean, Elanco Animal Health, Indianapolis, IN) is a β-adrenergic agonist that increases the rate and efficiency of muscle growth in pigs (Watkins et al., 1990). Dietary L-carnitine has been shown to increase pyruvate carboxylase and increase the conversion of fat to energy (Owen et al., 2001a). Administration of L-carnitine to humans before exercise resulted in increased pyruvate carboxylase activity and reduced lactic acid formation. L-carnitine administration favored aerobic processes resulting in more efficient performance (Vecchiet et al., 1990). Bertol et al. (2005) reported that pigs fed L-carnitine had reduced changes in blood pH when subjected to vigorous handling procedures and electrical prod stimulation. Because L-carnitine influences enzymes involved in pyruvate carboxylase, we hypothesized that it may be able to reduce the negative effects of stress from handling and transportation in commercial swine production. The objective of this study was to determine the interactive effects of dietary L-carnitine and RAC on the metabolic response of finishing pigs to handling.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee.

General

The experiments were conducted in late summer and early fall at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). Each experiment was approximately 90 d in duration and Exp. 2 initiated approximately 35 d after the start of Exp. 1. Pigs were housed in a modified open-front building with 50% solid concrete and 50% concrete slat flooring. The barn comprised 2 identical wings with 16 pens per side. There was a scale and holding pens connecting the 2 wings. Each 1.8- by 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) was used in the 2 experiments. Growth performance data were collected from all pigs, and handling and stress data were collected from subsample of 128 pigs. In each experiment, 192 pigs were blocked by BW and ancestry (initially 36 kg BW) in a split-split-plot design with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). There were 12 pigs (6 barrows and 6 gilts) 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 mg/kg) from 36 kg until the end of each experiment (118 kg). The basal diet was formulated to contain 1.20% total Lys 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 mg/kg) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg). In these experiments, pigs were fed 20 mg/kg 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 estimated nutrient requirements (NRC, 1998).

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

Item	Phase I [2]	Phase II[2]	Phase III[2]
Ingredient, %			
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[3]	0.15	0.15	0.15
Trace mineral premix[4]	0.15	0.15	0.15
Medication[5]	0.05	0.05	–
Corn starch[6]	0.05	0.05	0.15
L-Lys HCl	0.15	0.15	0.15
Calculated composition, %			
CP	19.67	16.92	16.92
Lys	1.20	1.00	1.00
Lys:calorie ratio, g/Mcal ME	3.18	2.65	2.20
ME, kcal/kg	3,311	3,318	3,325
Ca	0.70	0.65	0.61
Total P	0.64	0.60	0.55

[1]Diets were formulated to meet or exceed NRC (1998) requirement estimates.

[2]Phase I (36 to 54 kg BW); Phase II (54 to 86 kg BW); Phase III (86 to 118 kg BW).

[3]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 mg; vitamin B_{12}, 0.03 mg; riboflavin, 5.95 mg; pantothenic acid, 19.8 mg; and niacin, 33.1 mg.

[4]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.

[5]Provided 44 mg tylosin per kg diet.

[6]L-carnitine replaced cornstarch to provide 0 or 50 mg/kg carnitine in Phase I, II, and III. Ractopamine HCl (RAC; Elanco Animal Health, Indianapolis, IN) replaced cornstarch to provide 0 or 20 mg/kg RAC in Phase III.

Growth Performance

Body 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.

Stress Model

The 2 handling treatments (gentle and aggressive) were imposed at the end of the experiment (118 kg). Eight pigs from each diet (4 blocks and 2 pigs per pen) were 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 at a time). Two pigs from each pen were subjected to the gentle handling treatment, and 2 pigs from each pen were subjected to the aggressive handling treatment. Pigs were selected randomly from each pen. The 2 handling treatments were conducted consecutively to avoid circadian and ambient temperature bias. The tests began at approximately 0800 h and were concluded by 1330 h. The handling portion of the study was conducted in the other wing of the facility so that other pigs on trial did not become excited as the handling treatments were being conducted.

In the gentle handling treatment, the handler moved pigs at a moderate, walking pace 3 times through a 50-m course, including up and down a 15° loading ramp, by using a sorting board. 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. The time to complete the complete course took approximately 15 min with a range of 8 to 27 min.

In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° loading ramp. Panels divided the alleyway and narrowed it, resulting in crowding, at 1 end to simulate a single chute used in commercial loading and slaughter facilities. Pigs were subjected to three 1-s stimulations with an electrical prod (The Green One HS200; Hot-Shot, Savage, MN) each time around the course. Use of an electric prod provided short-term discomfort so that physiological and metabolic differences due to dietary treatment could be determined and provided the same level of stimulation to all pigs in that category. The time to complete the complete course took approximately 13 min with a range of 8 to 19 min. This treatment served as a model for the stress that some pigs might incur as they are loaded and transported to and in slaughter facilities.

Rectal temperature was recorded and blood was collected immediately (within 5 min) before and after handling in Exp. 1 and immediately after and 1 h after handling in Exp. 2 because there were no differences observed before handling in Exp. 1. Blood was collected via anterior vena cava puncture and samples were obtained as quickly as possible to prevent additional stress. Pigs were restrained for blood collection with a snout snare and quickly released after blood collection. Pigs were restrained for fewer than 30 s. Approximately 10 mL of blood was collected into heparin-coated glass tubes (Monoject; Tyco Healthcare Group, Mansfield, MA). Blood samples were immediately placed on ice and transported to the Kansas State University College of Veterinary Medicine to be analyzed for blood lactate dehydrogenase (**LDH**), lactate, pH, glucose, urea N, partial pressure of CO_2 (**pCO_2**), partial pressure of O_2 (**pO_2**), percentage of hemoglobin saturated with O_2 (**sO_2**), HCO_3^-, Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, and cortisol with an autoanalyzer (StatProfile CCX; Nova Biomedical, Waltham, MA). All analyses were conducted on whole blood. 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 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 numbers of 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-split-plot design by using the MIXED procedure (SAS Inst. Inc., Cary, NC) with handling (gentle or aggressive) as the whole plot and diets (0 or 50 mg/kg L-carnitine and 0 or 20 mg/kg RAC) as the subplots. In each experiment, there were 4 observations per treatment diet (pens) for growth performance. A subsample of individual pigs (4 pigs per pen; 2 for gentle and 2 for aggressive) was used for metabolic and physiological response data with 8 observations (pigs) per treatment. Least square means were calculated for each independent variable and separated with the PDIFF option of SAS. Differences were considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ and $P \leq 0.10$.

RESULTS AND DISCUSSION

Validation of the Handling Stress Model

The handling model is validated by the effect of aggressive vs. gentle handling on LDH, lactate, cortisol, glucose, rectal temperature, and blood pH. These differences indicate that the handling course was successful in eliciting differences between gently and aggressively handled pigs, which was 1 of the criteria used for its design. Gillis et al.

Table 2. Combined interactive effects between L-carnitine and ractopamine HCl (RAC) on growth performance of finishing pigs in Exp. 1 and 2[1,2]

	L-carnitine, mg/kg							
	0		50					
	RAC, mg/kg					Probability, $P <$		
Item	0	20	0	20	SED	RAC × L-carnitine	L-carnitine	RAC
Pre-RAC[3]								
ADG, kg	0.96	–	0.94	–	0.02	–	0.40	–
ADFI, kg	2.48	–	2.48	–	0.03	–	0.95	–
G:F	0.387	–	0.379	–	0.01	–	0.45	–
d 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	0.368	0.425	0.337	0.455	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	0.379	0.402	0.374	0.404	0.01	0.53	0.68	0.01

[1]In each experiment, a total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary RAC treatments (0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg).

[2]Values are means of 8 observations (pens) and 12 pigs per pen.

[3]Pre-RAC = 36 to 86 kg BW.

(2007) performed a study in which pigs were fed diets with and without RAC for 33 d and then subjected to either a low- or moderate-intensity handling treatment that simulated marketing (i.e., loading, transportation, unloading, lairage, and final drive processes) for 3 d. Their results also showed that pigs subjected to moderate-intensity handling had 54% greater LDH activity and increased serum lactate than pigs subjected to low-intensity handling. We also observed that pigs fed RAC were 1) more susceptible to an increase in LDH in both handling treatments and 2) had greater LDH 1 h after handling; these results indicate that pigs fed RAC took longer to return to prehandling baseline than control pigs.

Another contributing factor to the observed responses is that pigs fed RAC are leaner than counterparts not fed RAC. Increased muscling or leanness may also predispose the pig to greater physiological effects of stress (Ivers et al., 2002b).

Combined Growth Performance

The growth performance data from Exp. 1 and 2 were combined (Table 2). There was no effect 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 or main effects of L-carnitine for the growth performance criteria. For the overall finishing period (36 to 118 kg), there were no RAC × L-carnitine interactions observed for ADG, ADFI, or G:F or main effects of L-carnitine.

Previous studies showed that supplementing finishing diets with 50 mg/kg of L-carnitine increased ADG by 3.1% and G:F by 4.1% (James et al., 2013). Rekiel and Zackiewicz (2004) also showed that 50 mg/kg of supplemental L-carnitine improved ADG and G:F of finishing pigs. Pietruszka et al. (2009) observed increased growth performance as well as decreased cholesterol, triglycerides, and low-density lipoproteins when L-carnitine and Fe was added to the diet. In contrast, Owen et al. (2001a,b) and Bertol et al. (2005) observed no difference in growth performance, but Owen et al. (2001a,b) reported decreased backfat thickness of growing pigs fed diets supplemented with 50 or 125 mg/kg L-carnitine.

Pigs fed RAC had greater ($P < 0.01$) ADG and G:F than pigs not fed RAC. The improvement we observed in ADG and G:F for pigs fed RAC is consistent if not greater than results of a meta-analysis of 23 published studies on RAC use in finishing pigs (Apple et al., 2007), which showed that feeding 5, 10, or 20 mg/kg RAC improved ADG by 11 to 12% compared with untreated controls. Likewise, G:F of finishing pigs fed RAC increased by 10, 13.3, and 16.7% as dosage increased from 5 to 10 to 20 mg/kg RAC, respectively. Finally, pigs fed diets containing 10 and 20 mg/kg (but not 5) RAC were leaner and had greater percentages of fat-free lean than control pigs (Apple et al., 2007). These data confirm the growth-promoting effects of RAC in finishing pigs.

Blood Lactate Dehydrogenase

Lactate dehydrogenase is a cytoplasmic enzyme that catalyzes a reversible reaction that converts pyruvate to lactate at the end of anaerobic glycolysis (Horton et al., 1996). There are several isoenzymes of LDH; however, individual isoenzyme analysis requires special assays that are not widely available. Therefore, we analyzed only total LDH in our experiments. As expected, aggressive

handling increased blood LDH posthandling ($P < 0.01$; Exp. 1 and 2; Tables 3 and 4, respectively) and also 1 h posthandling ($P < 0.01$). The difference between pre- and posthandling LDH and also between post- and 1 h posthandling LDH were larger with aggressive handling than with gentle handling ($P < 0.01$). Ractopamine did not affect blood LDH prehandling but did increase blood LDH posthandling ($P < 0.01$, Exp. 1 and 2) and 1 h posthandling ($P < 0.01$). L-carnitine did not affect blood LDH.

An increase in LDH indicates muscle damage and red blood cell hemolysis (Stockham and Scott, 2002), and increased LDH may be a result of local or diffuse cell damage. In addition, during periods of intense activity, when ATP is primarily gained via anaerobic pathways, LDH and lactate concentrations will increase. Blood LDH has been used for decades as an indicator of porcine stress and our results are consistent with previous studies (Topel et al., 1968; Bickhardt et al., 1976; Anderson et al., 2002).

Pigs fed RAC were 1) more susceptible to an increase in LDH in both handling treatments ($P < 0.01$) and 2) had greater LDH 1 h after handling ($P < 0.01$), indicating that pigs fed RAC took longer to return to prehandling baseline than control pigs. Ractopamine-fed pigs are leaner than counterparts not fed RAC. Increased muscling or leanness is likely to predispose the pig to greater physiological effects of stress (Gillis et al., 2007). Pigs with high lean deposition rates (RAC, porcine somatotropin, or genetic selection) have greater proportions of white muscle fibers (Type IIb) and decreased proportions of red fibers (Beermann et al., 1990; Aalhus et al., 1990). White fibers have a greater glycolytic capacity, a reduced oxidative capacity, reduced capillary blood flow, and greater LDH, all of which could contribute to a greater level of lactic acid production (Ashmore et al., 1972). It is likely that pigs with enhanced glycolytic capacity are more susceptible to acute metabolic acidosis when exposed to greater levels of handling stress during the marketing process.

Dietary L-carnitine has been shown to increase pyruvate carboxylase in humans and pigs (Siliprandi et al., 1990; Vecchiet et al., 1990; Owen et al., 2001a). 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 LDH production in pig aggressively handled or fed RAC.

Blood Lactate

Pigs fed RAC had an increased ($P < 0.05$) pre- and posthandling (Exp. 1 and 2) lactate concentration compared with pigs not fed RAC. Lactate concentration was greatest posthandling for pigs aggressively handled and the difference in pre- and posthandling lactate concentration was greater ($P < 0.03$) in pigs fed L-carnitine. In Exp. 1 and 2, pigs aggressively handled as expected had a greater ($P < 0.01$) post- and 1 h posthandling lactate concentration than pigs gently handled. The difference between lactate concentration measured posthandling and 1 h posthandling was greater ($P < 0.01$) for aggressively handled pigs than for gently handled pigs. The difference (larger decrease) was greater because posthandling lactate concentration was much greater for pigs aggressively handled than for gently handled pigs and, therefore, had further to decrease to approach normal levels as pigs recovered from the aggressive handling.

When collected at exsanguination, blood lactate concentration has been shown to increase and have detrimental effects on pork quality such as increased drip loss and lighter color (Warriss et al., 1994; Hambrecht et al., 2005). Previous studies have demonstrated that aggressively handled pigs had greater lactate concentrations than gently handled pigs (Anderson et al., 2002; Ivers et al., 2002a,b; Peterson et al., 2009) and that these concentrations were positively related to the incidence of downer or nonambulatory pigs. Our results agree with these previous findings, which illustrates the importance of allowing pigs ample time to recover after delivery to slaughter facilities. It is interesting that pigs fed RAC had a greater prehandling lactate concentration than pigs not fed RAC. This may suggest that pigs fed RAC were in a partial acidotic state before handling. Pigs fed RAC also had a greater posthandling lactate concentration than pigs not fed RAC. Aggressively handled pigs fed RAC had the greatest lactate concentration and it remained greater 1 h posthandling. We did not observe differences in LDH for pigs fed added L-carnitine, and lactate concentration was not affected. Gillis et al. (2007) also observed that pigs fed 10 mg/kg of RAC tended to have greater serum lactate concentrations after the final drive compared with untreated controls whereas pigs fed 5 mg/kg of RAC were intermediate. The observed effects of animal handling on blood lactate concentrations emphasize the importance of using proper animal handling techniques when marketing finishing pigs fed RAC.

Blood Cortisol

There was a posthandling RAC × handling interaction ($P < 0.04$) for posthandling cortisol concentration in Exp. 1 but not in Exp. 2. Aggressively handled pigs had an increased posthandling cortisol concentration compared with gently handled pigs. Feeding RAC

Table 3. Interactive effects of L-carnitine, ractopamine HCl (RAC), and handling on stress criteria of finishing pigs (Exp. 1)[1]

	Handling[2]															
	Gentle[3]				Aggressive[4]					Probability, $P <$						
	L-carnitine, mg/kg															
	0		50		0		50									
	RAC, mg/kg															
Item	0	20	0	20	0	20	0	20	SED	RAC × L-carnitine × handling	RAC × L-carnitine	L-carnitine × handling	RAC × handling	L-carnitine	RAC	Handling
LDH,[5] U/L																
Prehandling	533	533	537	534	550	604	558	594	25.7	–	0.76	–	–	0.95	0.46	–
Posthandling	488	588	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																
Prehandling	2.39	3.61	2.23	2.31	2.10	2.85	2.03	2.91	0.26	–	0.35	–	–	0.17	0.01	–
Posthandling	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
Cortisol, ng/ml																
Prehandling	12.45	14.81	14.15	9.92	15.99	18.36	12.93	15.11	1.73	–	0.33	–	–	0.18	0.70	–
Posthandling	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	0.89	0.13	0.21	0.09	0.15	0.01
Glucose, mg/dL																
Prehandling	87.25	88.38	88.50	89.75	87.88	84.25	82.50	88.25	1.82	–	0.20	–	–	0.86	0.54	–
Posthandling	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 N, mg/dL																
Prehandling	15.75	13.63	15.13	15.63	15.00	12.38	13.38	12.75	1.13	–	0.31	–	–	0.98	0.29	
Posthandling	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
Rectal temperature, °C																
Prehandling	39.17	39.29	38.99	39.04	39.40	39.44	39.16	39.18	0.13	–	0.78	–	–	0.01	0.49	–
Posthandling	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

[1]A total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary ractopamine HCl treatments (RAC; 0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg).

[2]The 2 handling treatments (gentle and aggressive) 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 aggressive handling treatment. There were 2 pigs per pen per handling group. Values are means of 8 observations (pigs).

[3]In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15° loading ramp, using a sorting board at a moderate pace (walking). 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.

[4]In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° loading ramp. Panels divided the alleyway and narrowed, resulting in crowding, at 1 end to simulate a single chute to model commercial loading and slaughter facilities. Pigs were subjected to three 1-s stimulations, by an electrical prod, 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.

[5]LDH = lactate dehydrogenase.

increased cortisol concentration only when pigs were aggressively handled. Aggressively handled pigs had a greater ($P < 0.01$) cortisol concentration 1 h posthandling and a greater difference (increase; $P < 0.01$) between cortisol concentrations measured posthandling and 1 h posthandling than gently handled pigs. In Exp. 2, pigs fed L-carnitine had increased ($P < 0.02$) posthandling cortisol concentrations, but this was not the case posthandling in Exp. 1.

Short stressful events (i.e., direct handling, isolation, and transportation) are usually followed by an increase in stress hormones (von Borell, 2001). Cortisol concentration generally peaks at approximately 30 min after initiation of stress in pigs (Prunier et al., 2005). Downer pigs have increased cortisol concentrations compared with non-downer pigs (Anderson et al., 2002; Ivers et al., 2002a,b). To avoid causing stress and biasing the data, the duration between when pigs were initially snared and released was fewer than 30 s when blood was collected. It has been reported that snaring pigs and releasing them quickly does not affect cortisol concentration (Haussmann et al., 2000). Like other stress response criteria measured in this study, cortisol concentrations were greater for aggressively handled

Table 4. Interactive effects of L-carnitine, ractopamine HCl (RAC), and handling on stress criteria of finishing pigs (Exp. 2)[1]

	Handling[2]									Probability, $P <$						
	Gentle[3]				Aggressive[4]											
	L-carnitine, mg/kg															
	0		50		0		50									
	RAC, mg/kg															
Item	0	20	0	20	0	20	0	20	SED	RAC × L-carnitine × handling	RAC × L-carnitine	L-carnitine × handling	RAC × handling	L-carnitine	RAC	Handling
LDH,[5] U/L																
Posthandling	476	621	457	532	509	560	542	637	29.5	0.23	0.69	0.08	0.41	0.86	0.01	0.13
1 h posthandling	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																
Posthandling	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
1 h posthandling	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
Cortisol, ng/ml																
Posthandling	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
1 h posthandling	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
Glucose, mg/dL																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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
Rectal temperature, °C																
Posthandling	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
1 h posthandling	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

[1]A total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary ractopamine HCl treatments (RAC; 0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg).

[2]The 2 handling treatments (gentle and aggressive) 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 aggressive handling treatment. There were 2 pigs per pen per handling group. Values are means of 8 observations (pigs).

[3]In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15° loading ramp, using a sorting board at a moderate pace (walking). 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.

[4]In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° 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 three 1-s stimulations, by an electrical prod, 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.

[5]LDH = lactate dehydrogenase.

pigs, and cortisol concentration increased further for pigs fed RAC (Exp. 1).

Blood Glucose

Aggressively handled pigs had greater ($P < 0.01$; Exp. 1 and 2) glucose concentration posthandling and 1 h posthandling and a greater ($P < 0.01$) difference pre- and posthandling than gently handled pigs. The difference in glucose concentration between posthandling and 1 h posthandling was greater (more of a decrease; $P < 0.01$) for aggressively handled pigs than for gently handled pigs. Cortisol activity increases blood glucose concentration by stimulating gluconeogenesis and the breakdown of glycogen (Stockham and Scott, 2002). Benjamin et al. (2001) and Anderson et al. (2002) observed that aggressively handled pigs had greater

blood glucose concentrations than gently handled pigs, similar to our results.

In Exp. 2, pigs fed RAC had decreased ($P < 0.04$) 1 h posthandling blood glucose concentration suggesting a more rapid return to similar prehandling values observed in Exp. 1.

As for L-carnitine, Bertol et al. (2005) observed that pigs fed L-carnitine had decreased baseline glucose values than 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., 2001a). However, like in the present study, Pietruszka et al. (2009) observed no differences in blood glucose concentration between finishing pigs fed with or without L-carnitine.

Blood Urea Nitrogen

In Exp. 1, dietary treatment did not affect pre- or posthandling urea N concentration. However, aggressively handled pigs had a greater difference (greater increase; $P < 0.01$) in urea N concentration between pre- and posthandling.

In Exp. 2, a RAC × L-carnitine × handling interaction ($P < 0.04$) was observed for urea N concentration measured posthandling and 1 h posthandling. Pigs fed either RAC or L-carnitine had decreased urea N concentrations, and urea N concentration was lowest for pigs handled gently. Aggressively handled pigs had a greater urea N concentration posthandling and 1 h posthandling than gently handled pigs. Pigs fed RAC or L-carnitine had lower urea N concentration posthandling and 1 h posthandling than pigs not fed RAC or L-carnitine. The difference between urea N concentration measured posthandling and 1 h posthandling was less ($P < 0.01$) for aggressively handled pigs than for gently handled pigs.

The changes in blood urea N observed in Exp. 1 and 2 may be the result of increased muscle breakdown occurring from the stress of aggressive handling. However, pigs fed either RAC or L-carnitine had lower urea N concentrations posthandling and 1 h posthandling than untreated controls. Urea N concentration would be expected to be low in pigs fed RAC because of increased protein deposition (Webster et al., 2007). But Rincker et al. (2003) observed no differences in urea N in weanling pigs fed added L-carnitine.

Rectal Temperature

In Exp. 1 there was a trend for an RAC × handling interaction ($P < 0.06$) for the difference in rectal temperature between pre- and posthandling. In gently handled pigs feeding RAC resulted in very little change in rectal temperature between pre- and posthandling whereas in aggressively handled pigs it resulted in a greater change (greater increase) in rectal temperature. Pigs fed added L-carnitine had a lower ($P < 0.01$) prehandling rectal temperature; however, the changes were relatively minor in comparison with the changes observed due to aggressive handling. Unlike Exp. 1, there were no RAC × handling interactions observed for rectal temperature in Exp. 2. However, both RAC and aggressive handling resulted in higher rectal temperature compared with gentle handling or not feeding RAC. There was a RAC × L-carnitine interaction ($P < 0.02$) for posthandling rectal temperature and a trend for a RAC × L-carnitine interaction ($P < 0.06$) for rectal temperature at 1 h posthandling. Pigs fed RAC had a higher rectal temperature than pigs not fed RAC; however, rectal temperature was higher for pigs fed RAC and L-carnitine than for pigs fed RAC and not fed L-carnitine. Aggressively handled pigs had a higher ($P < 0.01$) rectal temperature posthandling and 1 h posthandling than gently handled pigs.

Pigs subjected to aggressive handling and electric prodding had increased skin temperature (Benjamin et al., 2001) and rectal temperature (Peterson et al., 2009). Brundige et al. (1998) evaluated the effects 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 postloading than hurdle-loaded pigs. In our experiment, prehandling rectal temperature was slightly lower for pigs fed L-carnitine; however, it is difficult to explain a mechanism for this observation. Aggressively handled pigs had a higher rectal temperature than gently handled pigs immediately posthandling (Exp. 1 and 2) and 1 h posthandling (Exp. 2). Pigs fed RAC also had a higher rectal temperature immediately posthandling and 1 h posthandling (Exp. 2) than pigs not fed RAC, and rectal temperature 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 2 handling treatments and pigs fed RAC.

Effects on Acid–Base Balance

There were no differences in prehandling blood pH as a result of dietary treatment (Table 5). However a RAC × handling interaction was observed for posthandling blood pH ($P < 0.01$) and the difference between pre- and posthandling blood pH ($P < 0.05$). In both cases, RAC had no effect on blood pH in gently handled pigs; however, feeding RAC reduced blood pH to a greater extent in aggressively handled pigs. L-carnitine had no effect on blood pH.

In Exp. 2, there were no RAC × handling interactions observed for posthandling blood pH; however, feeding

RAC and aggressive handling slightly decreased blood pH, but the effects were not additive (Table 6). By 1 h posthandling, pH of RAC-fed pigs returned to values similar to pigs not fed RAC, but aggressively handled pigs still had decreased blood pH compared with gently handled pigs. Therefore the difference between post- and 1 h posthandling was greater for either pigs fed RAC or those aggressively handled.

Downer pigs or those aggressively handled have been reported to have decreased blood pH (Anderson et al., 2002; Ivers et al., 2002a,b). Although the effects of RAC and aggressive handling were additive on blood pH in Exp. 1, they were independent in Exp. 2. This suggests that pigs fed RAC or aggressively handled may result in a state of metabolic acidosis.

Peterson et al. (2009) also observed lower blood pH in aggressively handled pigs than in gently handled pigs; however, blood pH was not different for pigs fed and not fed RAC. In the current study, blood pH at 1 h posthandling was similar for pigs fed and not fed RAC. Although blood pH of aggressively handled pigs was still lower 1 h posthandling, it was near blood pH levels of gently handled pigs. In comparison, blood lactate concentrations of aggressively handled pigs at 1 h posthandling were still fivefold greater than those of gently handled pigs. Bertol et al. (2005) reported that pigs fed L-carnitine had reduced changes in blood pH when subjected to vigorous handling procedures and electrical prod stimulation. In contrast, we observed that feeding L-carnitine did not affect blood pH immediately or 1 h posthandling.

Pigs fed RAC had greater ($P < 0.03$) prehandling pCO_2 concentration than pigs not fed RAC. There was no effect of treatment on pCO_2 concentration posthandling or for the difference between pre- and posthandling pCO_2 concentration. However, in Exp. 2, pigs aggressively handled or fed RAC had a decreased ($P < 0.03$) pCO_2 concentration 1 h posthandling than pigs gently handled or not fed RAC. The difference between pCO_2 concentration measured posthandling and 1 h posthandling was greater (decreased more; $P < 0.01$) for aggressively handled pigs than for gently handled pigs. L-carnitine had no effect on pCO_2 concentration. However, in Exp. 2, pigs fed L-carnitine had a decreased ($P < 0.05$) pCO_2 concentration posthandling than pigs not fed L-carnitine.

An increase in pCO_2 is an indicator of hypercapnia, which results from excess CO_2 in the blood (Stockham and Scott, 2002). In contrast, hypocapnia results from a decrease in pCO_2, which is due to a deficiency of CO_2 in the blood. The normal pulmonary process is related to acid–base balance. Expiration of CO_2 results in elimination of H^+. Because blood H^+ concentration is very low compared with the concentration of HCO_3^- (ratio ≈ 1:600,000), this process does not decrease HCO_3^- concentration unless there is excessive generation of H^+ (Stockham and Scott, 2002). Anderson et al. (2002) and Ivers et al. (2002b) reported that downer pigs had decreased pCO_2 compared with non-downer pigs. During metabolic acidosis, increased H^+ concentration stimulates respiration; the result is increased removal of CO_2 from pulmonary blood, and this decreases pCO_2. These results further support the idea that pigs aggressively handled or fed RAC were in a state of metabolic acidosis 1 h posthandling and that added L-carnitine did not alleviate the effects of stress.

Aggressively handled pigs had a greater ($P < 0.04$; Exp. 1 and 2) posthandling pO_2 concentration than gently handled pigs. There was a L-carnitine × handling interaction ($P < 0.05$) for the difference in pO_2 concentration between pre- and posthandling. Aggressively handled pigs had greater differences in pO_2 between pre- and posthandling pO_2 concentration than gently handled pigs. Among aggressively handled pigs, those fed L-carnitine had a greater increase in the difference in pO_2 concentration than those not fed L-carnitine.

In Exp. 2, pigs fed L-carnitine had a greater ($P < 0.03$) pO_2 concentration posthandling than pigs not fed L-carnitine; hence the difference between pO_2 concentration measured posthandling and 1 h posthandling tended to be greater (greater decrease; $P < 0.06$) among pigs fed L-carnitine. Aggressively handled pigs also had a greater difference in the change between post- and 1 h posthandling than for gently handled pigs ($P < 0.03$).

Decreased pO_2 is an indicator of hypoxemia, which results from deficiency of dissolved O_2 in blood. Posthandling pO_2 was greater for aggressively handled pigs, probably because these pigs had an increased respiration rate compared with gently handled pigs. In contrast, Bertol et al. (2005) reported that changes in blood pO_2 concentrations for pigs fed 5% soy oil and 150 mg/kg L-carnitine, after a standard handling procedure, were less than those for 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). There was no effect of treatment on posthandling sO_2 concentration or the difference between pre- and posthandling sO_2 concentration. There was no effect of treatment on sO_2 concentration 1 h posthandling or the difference between sO_2 concentrations measured posthandling and 1 h posthandling. In contrast, Bertol et al. (2005) observed that pigs fed L-carnitine had a decreased change between baseline and posthandling sO_2 percentage compared with pigs not fed L-carnitine.

In both Exp. 1 and 2, aggressively handled pigs or those fed RAC had decreased ($P < 0.01$) HCO_3^- concentration posthandling than gently handled pigs or those not fed RAC. One hour posthandling, aggressively

Table 5. Interactive effects of L-carnitine, ractopamine HCl (RAC), and handling on acid–base balance criteria of finishing pigs (Exp. 1)[1]

	Handling[2]									Probability, $P <$						
	Gentle[3]				Agressive[4]											
	L-carnitine, mg/kg															
	0		50		0		50									
	RAC, mg/kg															
Item	0	20	0	20	0	20	0	20	SED	RAC × L-carnitine × handling	RAC × L-carnitine	L-carnitine × handling	RAC × handling	L-carnitine	RAC	Handling
Blood pH																
Prehandling	7.39	7.37	7.40	7.40	7.41	7.43	7.40	7.39	0.01	–	0.81	–	–	0.20	0.40	–
Posthandling	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
pCO_2,[5] mmHg																
Prehandling	62.75	64.26	59.50	62.03	58.94	61.59	57.19	62.86	1.40	–	0.48	–	–	0.29	0.03	–
Posthandling	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,[6] mmHg																
Prehandling	40.31	40.00	44.78	38.64	53.19	49.05	40.04	41.30	3.90	–	0.98	–	–	0.26	0.55	–
Posthandling	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,[7] %																
Prehandling	68.93	67.18	76.04	64.59	76.68	72.18	71.36	70.29	2.72	–	0.57	–	–	0.81	0.09	–
Posthandling	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																
Prehandling	38.41	37.35	37.55	38.76	37.79	38.20	37.99	38.15	0.43	–	0.25	–	–	0.69	0.68	–
Posthandling	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																
Prehandling	147.38	147.50	146.75	146.38	145.50	147.25	146.63	147.25	0.45	–	0.37	–	–	0.73	0.25	–
Posthandling	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																
Prehandling	5.00	5.09	4.93	4.96	5.15	5.00	4.84	5.25	0.09	–	0.17	–	–	0.48	0.30	–
Posthandling	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																
Prehandling	104.25	102.75	103.88	102.75	103.50	102.50	102.88	102.63	0.39	–	0.47	–	–	0.57	0.02	–
Posthandling	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																
Prehandling	5.71	5.94	5.81	5.79	5.64	5.73	5.63	5.79	0.05	–	0.38	–	–	0.96	0.01	–
Posthandling	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																
Prehandling	0.97	0.93	0.90	0.92	0.95	0.92	0.93	0.91	0.01	–	0.07	–	–	0.02	0.03	–
Posthandling	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

[1]A total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary ractopamine HCl treatments (RAC; 0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg).

[2]The 2 handling treatments (gentle and aggressive) 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 aggressive handling treatment. There were 2 pigs per pen per handling group. Values are means of 8 observations (pigs).

[3]In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15° loading ramp, using a sorting board at a moderate pace (walking). 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.

[4]In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° 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 three 1-s stimulations, by an electrical prod, 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.

[5]pCO_2 = partial pressure of CO_2.

[6]pO_2 = partial pressure of O_2.

[7]sO_2 = hemoglobin saturated with O_2.

Table 6. Interactive effects of L-carnitine, ractopamine HCl (RAC), and handling on acid–base balance criteria of finishing pigs (Exp. 2)[1]

	Handling[2]									Probability, $P <$						
	Gentle[3]				Aggressive[4]											
	L-carnitine, mg/kg															
	0		50		0		50									
	RAC, mg/kg															
Item	0	20	0	20	0	20	0	20	SED	RAC × L-carnitine × handling	RAC × L-carnitine	L-carnitine × handling	RAC × handling	L-carnitine	RAC	Handling
Blood pH																
Posthandling	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
1 h posthandling	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
pCO_2,[5] mmHg																
Posthandling	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
1 h posthandling	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,[6] mmHg																
Posthandling	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
1 h posthandling	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,[7] %																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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																
Posthandling	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
1 h posthandling	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

[1]A total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary ractopamine HCl treatments (RAC; 0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg).

[2]The 2 handling treatments (gentle and aggressive) 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 aggressive handling treatment. There were 2 pigs per pen per handling group. Values are means of 8 observations (pigs).

[3]In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15° loading ramp, using a sorting board at a moderate pace (walking). 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.

[4]In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° 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 three 1-s stimulations, by an electrical prod, 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.

[5]pCO_2 = partial pressure of CO_2.

[6]pO_2 = partial pressure of O_2.

[7]sO_2 = hemoglobin saturated with O_2.

handled pigs still had decreased HCO_3^- compared with gently handled pigs ($P < 0.01$).

There were RAC × handling interactions ($P < 0.01$) observed for posthandling Na^+ and K^+ concentration as well as the difference ($P < 0.06$) pre- and posthandling in Exp 1. Pigs aggressively handled and fed RAC had a greater posthandling Na^+ concentration than pigs gently handled or not fed RAC. Yet in Exp. 2, the responses to RAC or aggressive handling were not additive. There were RAC × L-carnitine × handling interactions ($P < 0.04$) for Na^+ and K^+ concentrations 1 h posthandling, but the differences appear to be of little biological significance.

However, K^+ concentrations increased 1 h posthandling relative to posthandling for gently handled pigs; in aggressively handled pigs the effect was opposite, suggesting they had not yet returned to baseline values.

Several respiratory and nonrespiratory 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, hemoglobin, and other proteins such as albumin). Of the total buffering capacity, HCO_3^- contributes more than 20 mmol/L, and the nonbicarbonate buffers contribute less than 10 mmol/L (Stockham and Scott, 2002). Anderson et al. (2002) and Ivers et al. (2002b) reported that downer pigs had less HCO_3^- concentration than non-downer pigs, which was similar in the study herein. 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 a high-DCAD (+481 mEq/kg) or low-DCAD (+81 mEq/kg) diet. Ivers et al. (2002b) observed that there were fewer downer pigs when fed the high-DCAD diet and that HCO_3^- was greater for pigs fed the high-DCAD diet. Excess H^+ in metabolic acidosis leads to consumption or decreased concentration of HCO_3^-, which is used as a buffer. In our experiments, posthandling HCO_3^- concentration was decreased for pigs aggressively handled or fed RAC (Exp.1 and 2) or fed L-carnitine (Exp. 2). At 1 h posthandling, HCO_3^- concentration was still reduced for aggressively handled pigs, fed RAC, or fed L-carnitine; however, HCO_3^- concentration had increased from concentrations measured immediately posthandling. This result demonstrates that pigs recovered from the handling treatment. Added L-carnitine did not affect the change between pre- and posthandling HCO_3^-, which indicates that added L-carnitine did not alleviate or speed up the recovery of pigs aggressively handled or fed RAC.

Pigs fed RAC had decreased ($P < 0.02$) prehandling Cl^- concentration than pigs not fed RAC. Pigs aggressively handled had greater ($P < 0.01$) posthandling Cl^- concentrations (Exp. 1 and 2). There was an RAC × L-carnitine interaction ($P < 0.04$) for Cl^- concentration 1 h posthandling. Pigs fed either RAC or L-carnitine had a decreased Cl^- concentration 1 h posthandling than pigs not fed RAC or L-carnitine; however, pigs fed both RAC and L-carnitine had a greater Cl^- concentration than pigs not fed L-carnitine and RAC. Aggressively handled pigs had a greater ($P < 0.01$) difference (decrease) between Cl^- concentrations measured posthandling and 1 h posthandling than gently handled pigs suggesting a return to base line values within the 1 h period.

Pigs fed RAC had greater ($P < 0.01$) prehandling Ca^{2+} and decreased Mg^{2+} concentrations than pigs not fed RAC in Exp 1 but no differences posthandling. Handling and L-carnitine did not influence Ca^{2+} concentration in Exp. 1, yet aggressively handled pigs had greater Ca^{2+} and Mg^{2+} concentrations post- and 1 h posthandling than gently handled pigs. The differences were between post- and 1 h posthandling indicated that by 1 h posthandling, Ca^{2+} and Mg^{2+} concentrations increased in gently handled pigs but decreased in aggressively handled pigs ($P < 0.01$).

Cations and anions in biologic fluids are involved in acid–base balance. Strong cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) are considered bases because when they are added to extracellular fluid, if there is not a balancing shift of a strong ion (e.g., remove K^+ or add Cl^-), 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 they are added to extracellular fluid, if there is not a balancing shift of a strong ion (e.g., add Na^+ or remove lactate), H^+ shifts into the extracellular fluid to make it more acidic (Stockham and Scott, 2002). Acidosis is a condition in which 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^{2+}, and K^+ and decreased base excess compared with non-downer pigs. This is supported by observations from Ivers et al. (2002a,b). Our results are similar in that aggressively handled pigs had increased Na^+, decreased Cl^- (Exp. 1), and increased Na^+, K^+, and Ca^{2+} immediately posthandling compared with gently handled pigs. These results demonstrate the effect of aggressive handling on acid–base balance of pigs.

Heart Rate

In Exp. 1, aggressively handled pigs had increased ($P < 0.01$) average, maximum, and change in heart rate compared with gently handled pigs (Table 7). However, neither RAC nor L-carnitine affected heart rate. Benjamin et al. (2001) showed that aggressive handling and use of electric prodding increased heart rates of pigs compared with gentle handling. Moreover, Marchant-

Table 7. Interactive effects of L-carnitine, ractopamine HCl (RAC), and handling on heart rate of finishing pigs (Exp. 1)[1]

	Handling[2]															
	Gentle[3]				Aggressive[4]											
	L-carnitine, mg/kg									Probability, $P <$						
	0		50		0		50									
	RAC, mg/kg									RAC × L-carnitine × handling	RAC × L-carnitine	L-carnitine × handling	RAC × handling	L-carnitine	RAC	Handling
Item	0	20	0	20	0	20	0	20	SED							
No. of observations	6	8	5	7	6	6	4	4								
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 (maximum – minimum)	133	133	138	132	164	141	153	167	13.19	0.10	0.20	0.66	0.92	0.46	0.56	0.01

[1]A total of 192 pigs (initially 36 kg BW) was used with 2 handling treatments (whole plot) and 4 dietary treatments (subplots). Pigs were fed a corn–soybean meal diet with or without added L-carnitine (0 or 50 mg/kg) from 36 kg until the end of each experiment (118 kg). Dietary ractopamine HCl treatments (RAC; 0 or 20 mg/kg; Elanco Animal Health, Indianapolis, IN) were fed for the last 4 wk of the experiment (approximately 86 to 118 kg). Heart rate was only measured in Exp. 1 by using Polar Vantage NV heart rate monitor (Polar Electro Oy, Kempele, Finland).

[2]The 2 handling treatments (gentle and aggressive) 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 aggressive handling treatment. There were 2 pigs per pen per handling group. Values are means of 8 observations (pigs).

[3]In the gentle handling treatment, the handler moved pigs 3 times through a 50 m course, including up and down a 15° loading ramp, using a sorting board at a moderate (walking) pace. 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.

[4]In the aggressive handling treatment, pigs were moved as fast as possible through the course, including up and down a 30° loading ramp. Panels divided the alleyway and narrowed, resulting in crowding, at 1 end to simulate a single chute to model commercial loading and slaughter facilities. Pigs were subjected to three 1-s stimulations, by an electrical prod, 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.

Forde et al. (2003) demonstrated that feeding pigs RAC affected behavior, heart rate, and catecholamine profile. These pigs were more difficult to handle and susceptible to handling and transport stress.

In conclusion, this study demonstrates that dietary L-carnitine fed alone or in combination with RAC does not alleviate the handling effects of stress. This research emphasizes the importance of using proper animal handling techniques when marketing finishing pigs fed RAC.

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