

Hand-held lactate analyzer as a tool for the real-time measurement of physical fatigue before slaughter and pork quality prediction

L. M. Rocha^{1,2}, A. Dionne³, L. Saucier¹, E. Nannoni⁴ and L. Faucitano^{2†}

¹Department of Animal Science, Laval University, Quebec City, Canada, G1V 0A6; ²Agriculture and Agri-Food Canada, 2000 College Street, Sherbrooke, Canada, J1M 0C8; ³Olymel Fork, 568 Chemin de l'Écore, Vallée-Jonction, Canada, G0S 3J0; ⁴Department of Medical Veterinary Sciences, University of Bologna, Ozzano Emilia, 40064, Italy

(Received 18 February 2014; Accepted 28 August 2014; First published online 17 November 2014)

The objectives of this study were to assess the relationship between blood lactate variation measured at the plant, and pork quality variation on a large sample size and under commercial preslaughter handling conditions. A total of 600 pigs were randomly chosen on arrival at a commercial slaughter plant and blood samples taken from the ear vein at unloading (UN), after lairage (LA), in the restrainer (RE; before stunning) and at exsanguination (EX) were analysed for lactate content using a Lactate Scout Analyzer (LSA). In order to have a large range of measures, pigs were distributed into two groups; one kept in lairage overnight (G1) and the other for 2 to 3 h (G2) before slaughter. Meat quality was assessed in the Longissimus thoracis (LT), Semimembranosus (SM) and Adductor (AD) muscles by measuring the pH 30 min postmortem (pH1) and at 24 h postmortem (pHu), the colour and the drip loss. Blood lactate levels did not differ between G1 and G2 ($P > 0.05$). A reduced muscle lactate and glucose contents ($P = 0.02$ and $P = 0.004$, respectively) resulting in a lower ($P < 0.001$) glycolytic potential (GP) was observed in the LT muscle of G1 pigs when compared with G2 loins. In the LT muscle of G1 pigs, the lower GP resulted in an increased pHu ($r = -0.67$; $P < 0.001$), decreased drip loss ($r = 0.57$; $P < 0.001$) and darker colour ($r = 0.50$; $P < 0.001$) compared with G2. In both G1 and G2 pigs, the lower GP was correlated to higher pHu value in the SM and AD muscles ($r = -0.73$; $P < 0.001$). The greatest correlation was observed in G2 between blood lactate levels at LA and pHu value of the SM and AD muscles ($r = 0.46$ and $r = 0.44$, respectively; $P < 0.001$ for both muscles). The second greatest correlation was found between blood lactate levels at EX and pH1 value in the SM muscle in both groups ($r = -0.37$ and $r = -0.41$, respectively; $P < 0.001$ for both groups). Based on the results of this study, it appears that blood lactate levels, as measured by the LSA, reliably reflect the physiological response of pigs to perimortem stress and may help explain the variation in pork quality.

Keywords: stress, lactate, blood, meat quality, pigs

Implications

The majority of meat quality defects are directly related to preslaughter procedures, which are known to influence the physiological state of pigs before and at slaughter. Hence, the Lactate Scout Analyzer used in this study may be an accurate tool to assess the physiological condition of pigs under commercial conditions, and to predict the variation of meat quality traits. Furthermore, it may allow plant managers to identify critical points to be controlled in the preslaughter procedures in order to improve animal handling, facilities design, etc., and ultimately to limit meat quality losses.

Introduction

Muscular activity requires energy, which is provided by the breakdown of glycogen in the skeletal muscles. During intense muscular activity, the oxygen supply is often insufficient, so the energy is released through an anaerobic process, which converts pyruvate to lactate (Nelson and Cox, 2008). Therefore, lactate is either released into the blood flow in very disturbed or frightened animals or when there is some muscle damage (bruising), caused by vigorous physical exercise (Broom, 1995), and indicates an acidosis status of the pig as showed by the low blood pH (Ritter *et al.*, 2009). Earlier studies have associated greater values of exsanguination blood lactate to poor pork quality (Correa *et al.*, 2010; Edwards *et al.*, 2010). As blood lactate is not influenced by

[†] E-mail: luigi.faucitano@agr.gc.ca

post-stunning handling (Aalhus *et al.*, 1991) and is a very short-term stress indicator (higher peak in 4 min and return to basal levels in 2 h after physical exercise; Anderson, 2010), the greater lactate level in blood at slaughter definitely mirrors the physiological state of pigs before slaughter. For practical reasons, there is a need to develop a blood lactate measurement in the bleeding rail at the slaughter plant alternative to the traditional time-consuming enzymatic analytical procedure. The hand-held Lactate Scout Analyzer (LSA; EKF Diagnostic GmbH, Magdeburg, Germany) is being increasingly used for the measurement of blood lactate at swine slaughter plants, based on its strong correlation ($r = 0.97$; Edwards *et al.*, 2010) with the enzymatic procedure. This device would allow the monitoring of lactate variation in commercial conditions and assist in the development of improved animal handling methods before stunning. LSA blood lactate levels proved to be significantly correlated, although weakly, with a few pork quality traits, such as pH value 1 h *postmortem* and drip loss in the loin muscle (Edwards *et al.*, 2010). The low correlations reported in this study may be explained by the small sample size ($n = 128$ pigs), the low-stress handling conditions and by the fact that only the loin muscle was used for meat quality evaluation. There is evidence that the *longissimus* muscle may not be the most suitable muscle to study meat quality variation in relation to physical stress (Correa *et al.*, 2010).

Therefore, the objectives of this study were twofold: (1) to assess the relationship between lactate levels in blood collected at different points on the slaughter line and pork quality variation (in loins and hams) on a large sample size and under commercial preslaughter handling conditions and (2) to evaluate LSA's reliability as tool to prevent meat quality losses.

Material and methods

Animal ethics

All experimental procedures performed in this study were approved by the institutional animal care committee based on the current guidelines of the Canadian Council on Animal Care (2009).

Animals and treatments

In a 6-week trial, a total of 600 market-weight pigs (cross-breed F1 Yorkshire female \times Landrace sired with Duroc boar) were randomly chosen on arrival at a commercial slaughter plant (slaughter speed of 500 pigs/h; 7000 pigs/day) located in Eastern Canada over 6 slaughter days (1 day/week and 100 pigs/day). On each slaughter day, multiple trucks were randomly sampled to get 100 pigs (10% to 15% of total load/truck). Random selection of pigs was chosen in order to ensure the largest variation in pigs' physical conditions on arrival at the plant, with pre-transport fasting interval ranging from 6 to 12 h and transport time ranging from 0.5 to 5 h. Animals were identified by a numbered plastic ear tag to facilitate their identification at each sampling point and to track the carcasses for the meat quality assessment

after slaughter. Pigs were distributed into two main groups of 50 pigs each. The first group of 50 pigs was kept in one pen in lairage overnight (G1; $n = 300$), whereas the second group was kept in two pens, with 25 pigs each, and kept in lairage between 2 and 3 h before slaughter (G2; $n = 300$). In lairage, stocking density in the pen was $0.58 \text{ m}^2/\text{pig}$ for both groups. The stocking density in the lairage pen was controlled in this study as it may interfere, more than group size, on the effects of lairage time on pigs' resting behaviour (Moss, 1978). During lairage water was available through nipple type drinkers. Both lairage groups were sprinkled in the rest pen during the 30 to 45 min of end of lairage. Pigs were electrically stunned (head-to-chest electrical stunning) before exsanguination in the prone position.

Blood lactate analysis

Blood samples were collected from each pig by pricking one of the animal's distal ear veins with a retractable gauge needle. A drop of blood from the animal's ear was immediately dripped onto a sample strip (two strips or replicate/animal) and inserted into a hand-held LSA (EKF Diagnostic GmbH, Magdeburg, Germany), and the results were obtained in ~ 15 s. Pigs were sampled for lactate analysis at four different sampling points: at unloading (UN; $n = 600$), after lairage at the exit of the resting pen (LA; $n = 600$) and in the restrainer before stunning (RE; $n = 600$). The blood collection in the restrainer was carried out by stopping the restrainer for a few seconds right after the entrance of the animal into it. After electrical stunning, exsanguination blood was collected from the bleeding wound (EX; $n = 600$) in a plastic cup and lactate level was immediately assessed in duplicate with the LSA by dipping the test strips in the collected blood sample in order to collect $0.5 \mu\text{l}$ of blood in each strip. The bleeding wound was preferred for blood sampling at exsanguination instead of the ear based on the positive correlations between lactate content in the ear venous blood and that in the jugular venous and arterial blood ($r = 0.80$ and $r = 0.74$, respectively; $P < 0.001$ for both sampling locations) obtained in a preliminary study (unpublished results).

Meat quality measurements

Each slaughter week, 25 carcasses were selected from each lairage groups (50 carcasses/slaughter day; total of 300 carcasses) according to the blood lactate level at exsanguination with the objective to ensure a large range of blood lactate levels and meat quality traits. About 35 min after slaughter, carcasses were blast chilled (-20°C) for 90 min and then transferred to standard chilling rooms (3°C) where they were kept until the next day.

Meat quality was assessed in the *Longissimus thoracis* (LT; at the 3rd/4th last rib), *Semimembranosus* (SM; in the middle region) and *Adductor* (AD) muscles. Muscle pH was measured at 30 min *postmortem* (pH1) in the LT and in the SM muscles by means of a portable pH meter (Model pH 100 Series; Oakton Instruments, Vernon Hills, IL, USA) fitted with a Cole Parmer spear tip electrode (Cole Palmer Instrument Company, Vernon Hills, IL, USA) and an automatic temperature

compensation probe. This measurement was repeated at 24 h *postmortem* (pHu) in the same muscles and in the AD muscle. At 24 h *postmortem*, colour data were collected on the LT and SM muscles at the aforementioned anatomical locations after 30 min blooming time. Visual colour was evaluated using the Japanese color standards (Nakai *et al.*, 1975) in the LT muscle only, whereas instrumental colour (L^* , a^* and b^* values) was measured with a Minolta Chroma-meter (CR-300; Minolta Canada Inc., Mississauga, Canada) equipped with a 25-mm aperture, 0° viewing angle and D65 illuminant in the LT and SM muscles. Drip loss was measured in a LT muscle chop removed at the 3rd/4th last rib level and in the middle region of the SM muscle by a modified EZ-DripLoss procedure (Correa *et al.*, 2007). Briefly, three 25-mm diameter cores were removed from the centre of a 2.5-cm thick LT and SM muscle cross-section, weighed and placed into plastic drip loss containers (Christensen Aps Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4°C . At the end of the 48-h storage period, muscle cores were removed from their containers, surface moisture was carefully dabbed, cores were re-weighed and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight.

The floppiness score of the LT muscle was assessed by finger testing before dissection by a trained evaluator using a subjective scale ranging from 1 to 3 (1 = very soft and watery to 3 = very firm and dry; National Pork Board, 2000).

A sample of the LT muscle was also harvested in the region of the 3rd/4th last rib and immediately frozen in liquid nitrogen at 24 h *postmortem* for the analysis of the glycolytic potential (GP). The analysis was performed according to the method described by Monin and Sellier (1985) with some modifications and following the extraction protocol described by Bergmeyer *et al.* (1974). Briefly, 1 g of the LT muscle was homogenized in a Polytron device (System Polytron® PT 3100, Kinematica AG, Luzern, Switzerland) and then the samples were centrifuged at $2000 \times g$ for 20 min at 4°C . For the enzymatic determination of glycogen, glucose and glucose-6-Phosphate, 500 μl were transferred to glass tubes and the rest of homogenate was filtered with a filter paper (Whatman # 4; Buckinghamshire, UK) and the homogenate was kept at 4°C for the enzymatic determination of lactate. The samples were homogenized in buffer containing Rhizopus amyloglucosidase to decompose glycogen to glucose and glucose-6-phosphate. Lactate concentration in the homogenized samples was determined using NAD and lactate dehydrogenase. Glucose concentration was determined using a NAD, glucose-6-phosphate, ATP and enzymatic solution of hexokinase. The GP was quoted in terms of potential lactate formation according to the following formula proposed by Monin and Sellier (1985): $2 \text{ ([glycogen]} + \text{[glucose]} + \text{[glucose-6-phosphate]}) + \text{[lactate]}$. GP is expressed as $\mu\text{mole glucose equivalent/g}$ of fresh muscle.

Statistical analyses

All statistical procedures performed in the current study were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002). Blood lactate values

were log-transformed (\log_{10}) for data normalization before analysis. Log values were analysed for each sampling point with the MIXED procedure of SAS using sampling points as repeated measures in a one-way ANOVA for the group effect with the animal as the experimental unit and the week as random effect. Resulting adjusted means and confidence limits were back transformed to the original scale and used to build up Figure 1. Multiple comparisons between sampling points were adjusted with a Tukey–Kramer correction.

ANOVA for quality traits, potential glycolytic, muscle lactate and muscle glucose were carried out using the MIXED procedure of SAS. The model included the group as a fixed effect, the animal as the experimental unit and the week as a random effect. For variables showing a non-normal distribution of residuals, the analysis was performed with the non-parametric Wilcoxon Mann–Whitney test, using the NPAR1WAY procedure with the WILCOXON option. Spearman correlations were performed between blood lactate concentration at different sampling points and meat quality. Floppiness scores were analysed by the FREQ procedure of SAS using the Cochran–Mantel–Haenszel statistic to determine the effect of group on the mean score.

Results and discussion

Blood lactate variation

The physical activity associated with handling and fighting in lairage may cause physiological changes in pigs during the preslaughter period. As showed in Figure 1, in this study average lactate levels were of 3.66 mM (range: 3.50 to 3.83 mM) at unloading, dropped to 2.88 mM (range: 2.77 to 3.00 mM; $P < 0.001$) after resting in the lairage pen, regardless of the resting time and increased to 5.00 mM (range: 4.81 to 5.19 mM; $P < 0.001$) before stunning and to 8.71 mM (range: 8.37 to 9.08 mM) at exsanguination. The increase in blood lactate concentration between LA and RE reflects the progressively higher level of muscle activity and stress as the animals are handled and pass from a free-moving group situation to a single line of aligned and

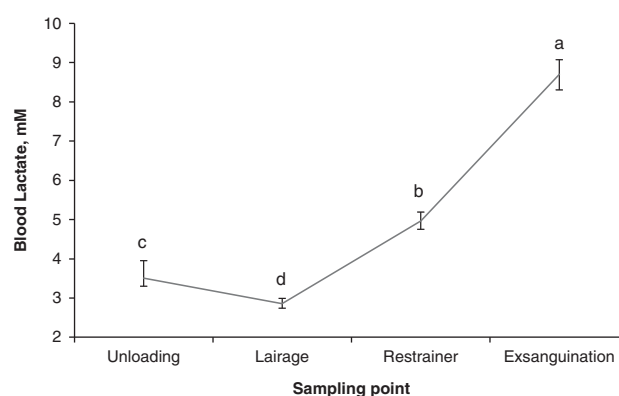


Figure 1 Preslaughter variation of blood lactate levels (mM; \pm confidence limits) collected in pigs at four different sampling points at the slaughter plant. Between sampling points, means with a different letter differ significantly ($P < 0.001$).

Table 1 Spearman correlations between lactate concentrations collected in pigs at four different sampling points at the slaughter plant

Sampling point R	UN	LA	RE	EX
UN	1.00	0.20***	0.17***	0.22***
LA		1.00	0.45***	0.23***
RE			1.00	0.60***
EX				1.00

UN = unloading ($n = 600$); LA = end of lairage ($n = 583$); RE = restrainer ($n = 581$); EX = exsanguination ($n = 583$).

*** $P < 0.001$.

restrained individuals. Other studies also reported increased blood concentration of lactate at exsanguination (Hunter *et al.*, 1994; Edwards *et al.*, 2011), and body temperature (Stewart *et al.*, 2005) in pigs being moved forward in a single line to the stunning point.

Based on the highest correlation between RE and EX blood lactate levels ($r = 0.60$; $P < 0.001$; Table 1), the measurement of blood lactate level using the LSA at the entrance into the restrainer appears to be the best indicator of physical fatigue of pigs at slaughter. However, our results also showed an increase in the blood lactate level between RE and EX ($P < 0.001$; Figure 1), meaning that electrical stunning may have an impact on the rate of lactate release into the blood flow at slaughter in this study. Greater blood lactate levels have also been reported in electrically v. gas stunned pigs by Bertoloni *et al.* (2006). This difference can be explained by the greater muscle contraction (tonic phase) in response to electrical current application.

Similarly to Edwards *et al.*'s study (2011), EX blood lactate levels as measured by the LSA in this study were lower than those reported by Hambrecht *et al.* (2004 and 2005) that reported lactate values ranging from 12 to 31 mM in exsanguination blood analysed with the traditional enzymatic procedure. The explanation for these differences between studies may be twofold: (1) the different distribution of lactate between whole blood (i.e. blood from which no constituent, such as red blood cells, white blood cells, plasma or platelets, has been removed according to the American Heritage® Science Dictionary, 2005) and plasma resulting in the underestimation of blood lactate concentrations when whole blood instead of plasma alone is used for analysis and (2) the difference in stress level (high v. minimal) experienced by pigs before slaughter in the two studies. Indeed, results obtained in a preliminary study showed that LSA is an efficient tool to detect pig fatigue after physical exercise based on the significant ($P > 0.001$) increase in blood lactate levels from rest to post-handling stress, that is, pigs were imposed to walk at a fast pace for 250 m (2.41 ± 0.84 v. 7.63 ± 3.98 mM, unpublished results).

According to Pösö and Puolanne (2005), blood lactate concentration may vary between 5 and 25 mM in meat animals. Furthermore, the distribution of lactate in blood does not appear to be homogenous (Harris and Dudley, 1989). For example, it was reported that whole blood lactate

is ~40% lower than plasma lactate concentration, although they are strongly correlated ($r = 0.993$; Foxdal *et al.*, 1990). The greater concentration of lactate in plasma compared with whole blood may explain the difference in lactate values reported by Hambrecht *et al.* (2004 and 2005) in blood plasma and those found in our study and in Edwards *et al.* (2010 and 2011) where lactate content was analysed by the LSA in the whole blood. The underestimation of the blood lactate content as measured with the LSA may be also explained by the significant delay of the transfer of lactate from plasma into red cells in the whole blood after it is generated in the muscle tissue until a balance is reached (Forrest *et al.*, 1990).

Considering the speed rate of lactate to reach the maximum concentration after stress in blood (4 min; Anderson, 2010), the stress level applied in the *perimortem* phase may be another possible explanation for the difference in blood lactate contents between this study and those reported in the literature. Greater lactate concentrations in exsanguination blood have been reported in pigs aggressively moved (use of electric prods and yells) to the stunner (Hambrecht *et al.*, 2004). Whereas, similarly to Edwards *et al.* (2010), in this study where the *perimortem* handling conditions were controlled (i.e. driving small groups without electric prods), the stress level applied on pigs before stunning does not appear to have been sufficient to produce an elevation of lactate levels in blood at exsanguination. Benjamin *et al.* (2001) also reported no variation in blood lactate concentration in pigs that were pushed to walk a long distance (300 m), but were handled gently (natural pace without electric prods).

Effect of lairage time on blood lactate concentration

Differently from Warriss *et al.* (1998) and Edwards *et al.* (2010) who reported greater exsanguination blood lactate levels in pigs after long lairage (overnight v. 4 h), blood lactate levels did not differ between lairage groups in this study, meaning that lairage time did not influence blood lactate concentration at slaughter (Table 2). Pérez *et al.* (2002) and Hambrecht *et al.* (2005) did not find significant effect on blood lactate concentration at exsanguination between long (up to 9 h) and short (<45 min) lairage groups either.

It is worth mentioning that blood lactate levels recorded at LA in this study were lower than 4 mM, which is the resting level of blood lactate reported for market-weight pigs in a previous study (Edwards *et al.*, 2011). Based on the speed of blood lactate level to return to rest level (120 min; Anderson, 2010), the low blood lactate levels after lairage recorded in this study would indicate that pigs had the adequate lairage conditions to recover from the stress of transport and unloading, regardless of the lairage time.

Meat quality

Effect of lairage time on meat quality traits

The purpose of lairage is to allow an opportunity for stressed and (or) fatigued animals to recover from loading and transport and to improve pork quality (Warriss, 2003). No difference

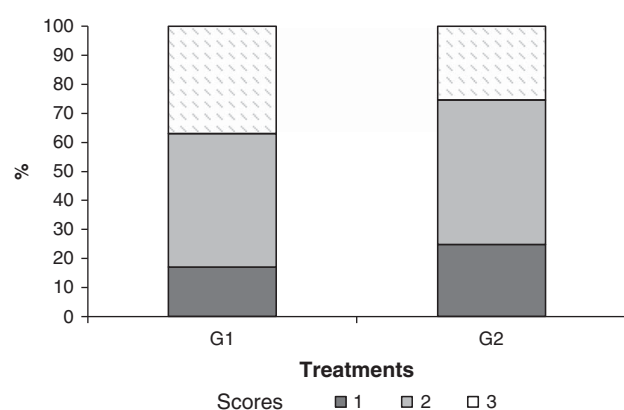
Table 2 Descriptive statistics of blood lactate levels (mM) per lairage group¹ of pigs at four sampling points

Sampling point	G1				G2			
	<i>n</i>	Mean	Lower	Upper	<i>n</i>	Mean	Lower	Upper
UN	300	3.65	3.44	3.87	300	3.64	3.43	3.86
LA	299	2.98	2.82	3.17	284	2.74	2.58	2.90
RE	297	4.98	4.69	5.28	285	4.96	4.67	5.27
EX	299	8.76	8.26	9.29	285	8.59	8.09	9.13

UN = unloading; LA = end of lairage; RE = restrainer; EX = exsanguination.

¹G1 = group kept in lairage overnight; G2 = group kept in lairage between 2 and 3 h.**Table 3** Variation of meat quality characteristics in the longissimus thoracis (LT), semimembranosus (SM) and adductor (AD) muscles of pigs according to the lairage group¹

Variable	G1			G2			<i>P</i> value ²
	<i>n</i>	Mean	s.d.	<i>n</i>	Mean	s.d.	
LT muscle							
pH1	133	6.64	0.22	156	6.63	0.20	NS
pHu	135	5.74	0.14	157	5.70	0.14	0.03
L*	134	51.26	3.54	157	52.39	3.26	0.002
Drip loss (%)	134	2.69	2.12	157	3.25	1.88	0.001
SM muscle							
pH1	132	6.81	0.18	156	6.79	0.21	NS
pHu	135	5.92	0.18	156	5.86	0.17	0.005
L*	135	49.52	2.94	156	50.35	2.82	0.002
Drip loss (%)	135	1.91	1.19	156	2.41	1.41	0.0009
AD muscle							
pHu	135	6.13	0.27	156	6.06	0.25	0.005

¹G1 = group kept in lairage overnight; G2 = group kept in lairage between 2 and 3 h.²Z-Wilcoxon test.**Figure 2** Comparison of scores frequency (from 1 to 3) for floppiness assessed by finger test in the longissimus thoracis (LT) muscle of pigs in two lairage groups. Floppiness scores: 1 = very soft and watery; 2 = normal and 3 = very firm and dry. G1 = group kept in lairage overnight; G2 = group kept in lairage between 2 and 3 h.

was observed in pH1 between lairage groups. As expected, compared with 2 to 3 h lairage (G2), overnight lairage (G1) resulted in a greater pHu in the LT, SM and AD muscles ($P = 0.03$, $P = 0.005$ and $P = 0.005$, respectively) and lower L^* and drip loss values in the LT and SM muscles

Table 4 Variation of lactate content, glucose content and glycolytic potential measured in the longissimus thoracis (LT) muscle of pigs from two lairage groups

	G1	G2	s.e.m.	<i>P</i> value
<i>n</i>	125	150		
Lactate ($\mu\text{mol/g}$) ¹	90.90	95.01	5.16	0.02
Glucose ($\mu\text{mol/g}$)	5.42	6.48	0.30	0.004
GP ($\mu\text{mol/g}$)	124.32	134.60	5.96	<0.001

GP = glycolytic potential.

¹All results are presented by $\mu\text{mol/g}$ of meat from the LT muscle at 24 h postmortem.

($P = 0.002$ for both muscles and $P = 0.001$ and $P < 0.001$, respectively; Table 3). Moreover, a greater ($P = 0.02$) proportion of firm and dry (score 3) loins was found in G1 loins compared with G2 (37.0% v. 25.5%; Figure 2). Increased incidence of greater pHu and darker and firmer pork after long lairage has been extensively reported in the literature (Warriss, 2003) and is explained by muscle glycogen depletion caused by extended feed restriction and muscle fatigue (Fernandez and Tornberg, 1991; Hambrecht *et al.*, 2004).

Overall, the GP values obtained in this study (Table 4) are within the range reported for the LT muscle of pigs in the

literature (128 to 154 $\mu\text{mol/g}$ fresh tissue; Przybylski *et al.*, 1994; Hambrecht *et al.*, 2004). However, similarly to meat quality traits, lairage time had an effect on the GP of the LT muscle, with muscle lactate and glucose contents and GP values being lower ($P = 0.02$, $P = 0.004$ and $P < 0.001$, respectively) in the LT muscle of pigs kept in lairage overnight (Table 4). Zhen *et al.* (2013) also reported decreased lactate and glucose concentrations and GP value in the LT muscle of

pigs as lairage time increased. The GP variation reflects the greater *antemortem* muscle energy exhaustion in the loin muscle of G1 pigs and contributes to explain the variation in pHu in the LT, SM and AD muscles ($r = -0.67$ and $r = -0.73$ for both SM and AD muscles, respectively; $P < 0.001$ for all muscles), in drip loss ($r = 0.57$; $P < 0.001$) and L^* value ($r = 0.50$; $P < 0.001$) in the LT muscle compared with G2 (Table 5). The correlation between GP of

Table 5 Spearman correlations between glycolytic potential and meat quality characteristics as assessed in the longissimus thoracis (LT), semi-membranosus (SM) and adductor (AD) muscles by lairage group^{1,2}

Parameters R	G1			G2		
	GP	Lactate	Glucose	GP ²	Lactate	Glucose
LT muscle						
pH1	-0.18*	-0.39***	0.10	-0.31***	-0.30***	-0.16
pHu	-0.67***	-0.48***	-0.53***	-0.45***	-0.20*	-0.56***
L*	0.50***	0.37***	0.35***	0.32***	0.01	0.43***
Drip loss	0.57***	0.38***	0.47***	0.15	0.13	0.01
SM muscle						
pH1	-0.21*	-0.44***	0.11	-0.20*	-0.23***	-0.12
pHu	-0.73***	-0.47***	-0.68***	-0.56***	-0.30***	-0.62***
L*	0.24***	0.18*	0.19*	0.27***	0.03	0.38***
Drip loss	0.46***	0.11	0.61***	0.39***	0.10	0.51***
AD muscle						
pHu	-0.73***	-0.46***	-0.68***	-0.35***	-0.13	-0.47***

GP = glycolytic potential.

¹G1 = group kept in lairage overnight; G2 = group kept in lairage between 2 and 3 h.

²LT muscle ($n = 124$ for G1 and $n = 148$ for G2); SM muscle ($n = 123$ for G1 and $n = 148$ for G2); AD muscle ($n = 125$ for G1 and $n = 148$ for G2).

* $P < 0.05$; *** $P < 0.001$.

Table 6 Spearman correlations between blood lactate level at different sampling points on the dressing line and meat quality characteristics as assessed in the longissimus thoracis (LT), semimembranosus (SM) and adductor (AD) muscles by lairage group^{1,2}

Parameters (r)	G1				G2			
	UN	LA	RE	EX	UN	LA	RE	EX
LT Muscle								
pH1	-0.06	0.01	-0.04	-0.23*	0.00	0.16	-0.01	-0.20*
pHu	0.04	0.24*	0.22*	0.19*	0.18*	0.29**	0.07	-0.02
L*	0.02	-0.11	-0.09	0.14	-0.14	-0.18*	0.03	0.14
Drip loss	-0.14	-0.16	-0.04	0.03	-0.17*	-0.09	0.11	0.18*
GP ($\mu\text{mol/g}$) ³	-0.14	-0.33***	-0.26***	-0.16	-0.19*	-0.19*	0.05	0.00
Lactate ($\mu\text{mol/g}$)	0.03	-0.13	-0.16	-0.02	0.07	-0.11	0.06	-0.01
Glucose ($\mu\text{mol/g}$)	-0.26***	-0.30***	-0.27***	-0.29***	-0.41***	-0.19*	-0.04	-0.05
SM Muscle								
pH1	-0.18*	0.06	0.06	-0.37***	-0.09	0.13	-0.08	-0.41***
pHu	0.23*	0.28**	0.26**	0.29**	0.33***	0.46***	0.07	-0.07
L*	-0.18*	-0.02	-0.08	0.10	-0.22*	-0.26**	-0.04	0.17*
Drip loss	-0.23*	-0.21*	-0.19*	-0.09	-0.24**	-0.27**	0.01	0.22*
AD Muscle								
pHu	0.25**	0.29**	0.32**	0.28**	0.30**	0.44***	0.28**	0.13

UN = unloading; LA = end of lairage; RE = restrainer; EX = exsanguination; GP = glycolytic potential.

¹G1 = group kept in lairage overnight; G2 = group kept in lairage between 2 and 3 h.

²LT muscle ($n = 134$ for G1 and $n = 156$ for G2); SM muscle ($n = 133$ for G1 and $n = 155$ for G2); AD muscle ($n = 135$ for G1 and $n = 156$ for G2).

³GP ($n = 124$ for G1 and $n = 148$ for G2).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

the LT muscle and pHu in the SM muscle is not surprising as these muscles have comparable metabolic characteristics (Laborde *et al.*, 1985; Monin *et al.*, 1987). Indeed, similarly to the LT muscle, in the SM muscle pHu variation follows a curvilinear regression when GP increases ($r = -0.80$; $P < 0.001$; Przybylski *et al.*, 1994).

Correlations between blood lactate levels and meat quality

Spearman correlations between blood lactate concentration at different sampling points and meat quality traits by lairage event are showed in Table 6. Similarly to Edwards *et al.* (2010), in this study the correlations between blood lactate levels and meat quality traits in the LT muscle were generally low for both lairage groups. The greatest correlation was found between blood lactate level recorded at the end of the resting period when exiting the lairage pen (LA) and the pHu value in the SM and AD muscles ($r = 0.46$; $P < 0.001$ and $r = 0.44$; $P < 0.001$, respectively) in the G2 group.

The second greatest correlation was found between blood lactate levels at EX and pH taken at 1 h post-slaughter in the SM muscle in both G1 and G2 groups ($r = -0.37$ and $r = -0.41$, respectively; $P < 0.001$ for both lairage groups), suggesting a decreased pH1 as blood lactate levels increase at exsanguination. The contribution of exsanguination blood lactate levels to early *postmortem* acidification rate found in this study confirms what was already reported in previous studies (Hambrecht *et al.*, 2005; Edwards *et al.*, 2010). However, the correlations obtained in this study are greater than those reported by Edwards *et al.* (2010) using the LSA and the LT muscle as meat quality indicator ($r = -0.32$).

Overall, the greater correlations between blood lactate levels and meat quality traits in the ham muscles are not surprising as they are locomotor muscles and thus more prone to rapid glycogen exhaustion after physical exercise rather than postural muscles, such as the LT muscle. These results, similar to others from previous studies (Hambrecht *et al.*, 2005; Correa *et al.*, 2010), show that the effects of a specific stress on meat quality, either physical or psychological, are muscle dependent.

Conclusions

Overall, our results suggest that the hand-held scout analyzer is capable of measuring blood lactate levels variation associated with the physiological condition of pigs in the *perimortem* phase. However, although significant, the magnitude of the correlations between blood lactate and meat quality traits found in this study is rather low, meaning a poor reliability in predicting pork quality variation. Possible reasons for these low correlations can be either the small range of variation in the preslaughter stress levels applied in this study or the use of whole blood for lactate analysis resulting in an underestimation of lactate concentrations in blood. Thus, for a more reliable validation of the LSA

technique for the monitoring of the preslaughter conditions and control of pork quality variation, further studies in which the LSA is used as stand-alone measurement or in combination with other non-invasive tools (e.g. IR thermography) under more variable preslaughter conditions are needed.

Acknowledgements

The authors appreciate the assistance of S. Horth, R. Formighieri and P. Mattanna for the blood sampling, meat quality measurements and laboratory analysis. Special thanks go to S. Méthot for his expertise with the statistical analysis. The authors are grateful to Olymel Fork® for the financial support and for providing the slaughter facilities and the manpower to conduct this project.

References

- Aalhus JL, Gariépy C, Murray AC, Jones SDM and Tong AKW 1991. Stunning and shackling influences on quality of porcine longissimus dorsi and semimembranosus muscles. *Meat Science* 29, 323–334.
- American Heritage® Science Dictionary 2005. The American Heritage® Science Dictionary, 1st edition. Houghton Mifflin Company, Boston, MA, USA.
- Anderson DB 2010. Relationship of blood lactate and meat quality in market hogs. Presentation at the 63rd Reciprocal Meat Conference, Lubbock, TX. Retrieved 01 October 2012, from <http://fass.acrobat.com/p86799506/>.
- Benjamin ME, Gonyou HW, Ivers DL, Richardson LF, Jones DJ, Wagner JR, Seneriz R and Anderson DB 2001. Effect of handling method on the incidence of stress response in market swine in a model system. *Journal of Animal Science* 79, 279.
- Bergmeyer HU, Bern E, Schmidt F and Stork H 1974. D-Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of enzymatic analysis* (ed. HU Bergmeyer), pp. 1196–1201. Academic Press, New York, NY, USA.
- Bertoloni W, Silveira ETF, Ludtke CB and Costa MR 2006. Avaliação de diferentes híbridos suínos submetidos à insensibilização elétrica e gasosa (CO₂): parte 2 – mensurações objetivas de qualidade. *Ciência e Tecnologia de Alimentos* 26, 555–563.
- Broom DM 1995. Quantifying pig welfare during transport using physiological measures. Proceedings of the eu seminar 'New Information on Welfare and Meat Quality of Pigs as Related to Handling, Transport and Lairage Conditions', Mariensee, Germany, June 29–30, pp. 3–10.
- Canadian Council on Animal Care 2009. Guidelines on: the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, Canada.
- Correa JA, Méthot S and Faucitano L 2007. A modified meat juice container (EZ-DripLoss) procedure for a more reliable assessment of drip loss and related quality changes in pork meat. *Journal of Muscle Foods* 18, 67–77.
- Correa JA, Torrey S, Devillers N, Laforest JP, Gonyou HW and Faucitano L 2010. Effects of different moving devices at loading on stress response and meat quality in pigs. *Journal of Animal Science* 88, 4086–4093.
- Edwards LN, Engle TE, Correa JA, Paradis MA, Grandin T and Anderson DB 2010. The relationship between exsanguination blood lactate concentration and carcass quality in slaughter pigs. *Meat Science* 85, 435–440.
- Edwards LN, Engle TE, Grandin T, Ritter MJ, Sosnicki A, Carlson BA and Anderson DB 2011. The effects of distance traveled during loading, lairage time prior to slaughter, and distance traveled to the stunning area on blood lactate concentration of pigs in a commercial packing plant. *The Professional Animal Scientist* 27, 485–491.
- Fernandez X and Tornberg E 1991. A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. *Journal of Muscle Foods* 2, 209–235.
- Forrest AR, Morton S and Lambardarios C 1990. Blood or plasma lactate? *British Journal of Sports Medicine* 24, 132.
- Foxdal P, Sjödin B, Rudstam H, Östman C, Östman B and Hedenstierna GC 1990. Lactate concentration differences in plasma, whole blood, capillary finger blood and erythrocytes during submaximal graded exercise in humans. *European Journal of Applied Physiology and Occupational Physiology* 61, 218–222.

- Hambrecht E, Eissen JJ, Newman DJ, Smits CHM, Verstegen MWA and Den Hartog LA 2005. Negative effects of stress immediately before slaughter on pork quality are aggravated by suboptimal transport and lairage conditions. *Journal of Animal Science* 83, 440–448.
- Hambrecht EJ, Eissen J, Nooijen RIJ, Ducro BJ, Smits CHM, Den Hartog LA and Verstegen MWA 2004. Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. *Journal of Animal Science* 82, 1401–1409.
- Harris R and Dudley G 1989. Exercise alters the distribution of ammonia and lactate in blood. *Journal of Applied Physiology* 66, 313–317.
- Hunter EJ, Weeding CM, Guise HJ, Abbott RH and Penny RHC 1994. Pig welfare and carcass quality – a comparison of the influence of slaughter handling systems at two abattoirs. *Veterinary Record* 29, 423–425.
- Laborde D, Talmant A and Monin G 1985. Activités enzymatiques métaboliques et contractiles de 30 muscles du porc. Relations avec le pH ultime atteint après la mort. *Reproduction Nutrition et Développement* 25, 619–628.
- Monin G and Sellier P 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate *postmortem* period: the case of the Hampshire breed. *Meat Science* 13, 49–63.
- Monin G, Mejenes-Quijano A, Talmant A and Sellier P 1987. Influence of breed and muscle metabolic type on muscle glycolytic potential and meat pH in pigs. *Meat Science* 20, 149–158.
- Moss BW 1978. Some observations on the activity and aggressive behaviour of pigs when penned prior to slaughter. *Applied Animal Ethology* 4, 323–339.
- Nakai H, Saito F, Ikeda T, Ando S and Komatsu A 1975. Standard models of pork color. *Bulletin of National Institute of Animal Industry* 29, 69–74.
- National Pork Board 2000. Pork composition and quality assessment procedures. National Pork Board, Des Moines, IA, USA.
- Nelson DL and Cox MM 2008. *Lehninger principles of biochemistry*, 5th edition WH Freeman and Company, New York, NY, USA.
- Pérez MP, Palacio J, Santolaria MP, Aceña MC, Chacón G, Verde MT, Calvo JH, Zaragoza P, Gascón M and Garcia-Belenguér S 2002. Influence of lairage time on some welfare and meat quality parameters in pigs. *Veterinary Research* 33, 239–250.
- Pösö AR and Puolanne E 2005. Carbohydrate metabolism in meat animals. *Meat Science* 70, 423–434.
- Przybylski W, Vernin P and Monin G 1994. Relationship between glycolytic potential and ultimate pH in bovine, porcine and ovine muscles. *Journal of Muscle Foods* 5, 245–255.
- Ritter MJ, Ellis M, Berry NL, Curtis SE, Anil L, Benjamin M, Butler D, Dewey C, Driessen B, DuBois P, Hill J, Marchant-Forde J, Matzat P, McGlone JJ, Mormede P, Moyer T, Pfalzgraf K, Salak-Johnson J, Sterle J, Stull C, Whiting T, Wolter B, Niekamp SR and Johnson AK 2009. Transport losses in market weight pigs: in a review of definitions, incidence and economic impact. *The Professional Animal Scientist* 25, 404–414.
- SAS 2002. SAS – Statistical analysis system. Release 9.1. SAS Institute Inc., Cary, NC, USA.
- Stewart M, Webster JR, Schaefer AL, Cook NJ and Scott SL 2005. Infrared thermography as a non-invasive tool to study animal welfare. *Animal Welfare* 14, 319–325.
- Warriss PD 2003. Optimal lairage times and conditions for slaughter pigs: a review. *Veterinary Record* 153, 170–176.
- Warriss PD, Brown SN, Edwards JE and Knowles TG 1998. Effects of lairage time on levels of stress and meat quality in pigs. *Animal Science* 66, 255–261.
- Zhen S, Liu Y, Li X, Ge K, Chen H, Li C and Ren F 2013. Effects of lairage time on welfare indicators, energy metabolism and meat quality of pigs in Beijing. *Meat Science* 93, 287–291.