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

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Short title: Blood lactate and pork quality

Abstract

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 2-3 h (G2) before slaughter. Meat quality was assessed in the *Longissimus thoracis* (LT), S*emimembranosus* (SM) and *Adductor* (AD) muscles by measuring the pH 30 min *post-mortem* (pH1) and at 24 h *post-mortem* (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 to 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 to 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 *peri-mortem* 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*., 2010a, b). As blood lactate is not influenced by post-stunning handling (Aalhus *et al*., 2001) 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 prior to 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) 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*., 2010a) 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 *post-mortem* and drip loss in the loin muscle (Edwards *et al.,* 2010a). The low correlations reported in this

78 study may be explained by the small sample size (n = 128 pigs), the low-stress handling
79 conditions and by the fact that only the loin muscle was used for meat quality evaluation.
80 There is evidence that the *longissimus* muscle may not be the most suitable muscle to
81 study meat quality variation in relation to physical stress (Correa *et al.,* 2010).

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

87

88 **Materials and methods**

89 *Animal ethics*

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

93

94 *Animals and treatments*

95 In a 6 week trial, a total of 600 market weight pigs (crossbreed F1 Yorkshire female ×
96 Landrace sired with Duroc boar) were randomly chosen on arrival at a commercial
97 slaughter plant (slaughter speed of 500 pigs/hour, totalling 7,000 pigs/day) located in
98 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 - 15% of total load/truck). 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 2 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 m^2/ 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 last 30 – 45 min of lairage. Pigs were electrically stunned (head-to-chest electrical stunning) prior to 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 Lactate Scout Analyzer (LSA; EKF Diagnostic GmbH, Magdeburg, Germany), and the results were obtained in approximately 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 µl 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, twenty-five (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 $3^{rd}/4^{th}$ last rib), *Semimenbranosus* (SM; in the middle region) and *Adductor* (AD) muscles. Muscle pH was measured at 30 min *post-mortem* (pH1) in the LT and in the SM muscles by means of a portable pHmeter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted

143 with a Cole Parmer spear tip electrode (Cole Palmer Instrument Company, Vernon Hills,
144 IL) and an automatic temperature compensation (ATC) probe. This measurement was
145 repeated at 24 h *post-mortem* (pHu) in the same muscles and in the AD muscle. At 24 h
146 *post-mortem*, colour data were collected on the LT and SM muscles at the afore-
147 mentioned anatomical locations after 30 min blooming time. Visual color was evaluated
148 using the Japanese color standards (Nakai *et al.,* 1975) in the LT muscle only, whereas
149 instrumental colour (L*, a* and b* values) was measured with a Minolta Chromameter
150 (CR-300; Minolta Canada Inc., Mississauga, Canada) equipped with a 25-mm aperture,
151 0º viewing angle, and D65 illuminant in the LT and SM muscles. Drip loss was measured
152 in a LT muscle chop removed at the $3^{rd}/4^{th}$ last rib level and in the middle region of the
153 SM muscle by a modified EZ-Dripless procedure (Correa *et al*., 2007). Briefly, three 25
154 mm diameter cores were removed from the center of a 2.5 cm thick LT and SM muscle
155 cross-section, weighed, and placed into plastic drip loss containers (Christensen Aps
156 Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4°C. At the end
157 of the 48 h storage period, muscle cores were removed from their containers, surface
158 moisture was carefully dabbed, cores were re-weighed, and drip loss percentage was
159 calculated by dividing the difference between initial and final core weights by the initial
160 core weight.

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

164 A sample of the LT muscle was also harvested in the region of the $3^{rd}/4^{th}$ last rib and
165 immediately frozen in liquid nitrogen at 24 h *post-mortem* 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 (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 2,000 x g for 20 min at 4°C. For the enzymatic determination of glycogen, glucose and glucose-6-P, 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 nicotinamide adenine dinucleotide (NAD) and lactate dehydrogenase. Glucose concentration was determined using a NAD, glucose-6-phosphate, adenosine triphosphate (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 ([glycogen] + [glucose] + [glucose 6 phosphate]) + [lactate]. GP is expressed as µmole 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 (log10) for data normalization before analysis. Log values were

188 analyzed for each sampling point with the MIXED procedure of SAS using sampling
189 points as repeated measures in a one-way analysis of variance for the group effect with
190 the animal as the experimental unit and the week as random effect. Resulting adjusted
191 means and confidence limits were back-transformed to the original scale and used to
192 build up Figure 1. Multiple comparisons between sampling points were adjusted with a
193 Tukey-Kramer correction.

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

203

204 **Results and discussion**

205 *Blood lactate variation*

206 The physical activity associated with handling and fighting in lairage may cause
207 physiological changes in pigs during the preslaughter period. As showed in Fig. 1, in this
208 study average lactate levels were of 3.66 mM (ranging from 3.50 to 3.83 mM) at

209 unloading, dropped to 2.88 mM (range: 2.77 to 3.00 mM; $P < 0.001$) after resting in the
210 lairage pen, regardless of the resting time, and increased to 5.00 mM (range: 4.81 to
211 5.19 mM; $P < 0.001$) prior to stunning and to 8.71 mM (ranging from 8.37 to 9.08 mM) at
212 exsanguination. The increase in blood lactate concentration between LA and RE reflects
213 the progressively higher level of muscle activity and stress as the animals are handled
214 and pass from a free-moving group situation to a single line of aligned and restrained
215 individuals. Other studies also reported increased blood concentration of lactate at
216 exsanguination (Hunter *et al.,* 1994; Edwards *et al*., 2011), and body temperature
217 (Stewart *et al*., 2005) in pigs being moved forward in a single line to the stunning point.

218 Based on the highest correlation between RE and EX blood lactate levels ($r = 0.60$; $P <$
219 0.001; Table 1), the measurement of blood lactate level using the LSA at the entrance
220 into the restrainer appears to be the best indicator of physical fatigue of pigs at
221 slaughter. However, our results also showed an increase in the blood lactate level
222 between RE and EX ($P < 0.001$; Fig. 1), meaning that electrical stunning may have an
223 impact on the rate of lactate release into the blood flow at slaughter in this study.
224 Greater blood lactate levels have been also reported in electrically *vs.* gas stunned pigs
225 by Bertoloni *et al.* (2006). This difference can be explained by the greater muscle
226 contraction (tonic phase) in response to electrical current application.

227 Similarly to Edwards *et al.* (2011), EX blood lactate levels as measured by the LSA in
228 this study were lower than those reported by Hambrecht *et al*. (2004, 2005) which
229 reported lactate values ranging from 12 to 31 mM in exsanguination blood analyzed with
230 the traditional enzymatic procedure. The explanation for these differences between
231 studies may be two-fold: 1) the different distribution of lactate between whole blood (*i.e.*

232 blood from which no constituent, such as red blood cells, white blood cells, plasma, or
233 platelets, has been removed according to the American Heritage® Science Dictionary,
234 2005) and plasma resulting in the underestimation of blood lactate concentrations when
235 whole blood instead of plasma alone is use for analysis and 2) the difference in stress
236 level (high vs. minimal) experienced by pigs prior to slaughter in the two studies. Indeed,
237 results obtained in a preliminary study showed that LSA is an efficient tool to detect pig
238 fatigue after physical exercise based on the significant (P > 0.001) increase in blood
239 lactate levels from rest to post-handling stress, i.e. pigs were imposed to walk at a fast
240 pace for 250 m (2.41 ± 0.84 mM *vs.* 7.63 ± 3.98 mM, unpublished results).

241 According to Pösö and Puolanne (2005), blood lactate concentration may vary between
242 5 and 25 mM in meat animals. Furthermore, the distribution of lactate in blood does not
243 appear to be homogenous (Harris and Dudley, 1989). For example, it was reported that
244 whole blood lactate is approximately 40 % lower than plasma lactate concentration,
245 although they are strongly correlated (r = 0.993; Foxdal *et al*., 1990). The greater
246 concentration of lactate in plasma compared to whole blood may explain the difference
247 in lactate values reported by Hambrecht *et al.* (2004, 2005) in blood plasma and those
248 found in our study and in Edwards *et al.* (2010a,b, 2011) where lactate content was
249 analyzed by the LSA in the whole blood. The underestimation of the blood lactate
250 content as measured with the LSA may be also explained by the significant delay of the
251 transfer of lactate from plasma into red cells in the whole blood after it is generated in
252 the muscle tissue until a balance is reached (Forrest *et al.*, 1990).

253 Considering the speed rate of lactate to reach the maximum concentration after stress in
254 blood (4 min; Anderson, 2010), the stress level applied in the *peri-mortem* 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 *peri-mortem* handling conditions were controlled (i.e. driving small groups without electric prods), the stress level applied on pigs prior to 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*. (2010a) who reported greater exsanguination blood lactate levels in pigs after long lairage (overnight *vs.* 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 previous studies (Benjamin *et al.,* 2001; Edwards *et al*., 2011). Based on the speed of

277 blood lactate level to return to rest level (120 min; Anderson, 2010), the low blood
278 lactate levels after lairage recorded in this study would indicate that pigs had the
279 adequate lairage conditions to recover from the stress of transport and unloading,
280 regardless of the lairage time.

281

282 **Meat quality**

283 *Effect of lairage time on meat quality traits*

284 The purpose of lairage is to allow an opportunity for stressed and (or) fatigued animals
285 to recover from loading and transport and to improve pork quality (Warriss, 2003). No
286 difference was observed in pH1 between lairage groups. As expected, compared to 2-3
287 h lairage (G2), overnight lairage (G1) resulted in a greater pHu in the LT, SM and AD
288 muscles (P = 0.03, P = 0.005 and P = 0.005, respectively) and lower L* and drip loss
289 values in the LT and SM muscles (P = 0.002 for both muscles and P = 0.001 and P <
290 0.001, respectively; Table 3). Moreover, a greater (P = 0.02) proportion of firm and dry
291 (score 3) loins was found in G1 loins compared with G2 (37.0 *vs*. 25.5 %; Fig. 2).
292 Increased incidence of greater pHu and darker and firmer pork after long lairage has
293 been extensively reported in the literature (Warriss, 2003) and is explained by muscle
294 glycogen depletion caused by extended feed restriction and muscle fatigue (Fernandez
295 and Tornberg, 1991; Hambrecht *et al*., 2004).

296 Overall, the GP values obtained in this study (Table 4) are within the range reported for
297 the LT muscle of pigs in the literature (128-154 µmol/g fresh tissue; Przybylski *et al.*,
298 1994; Hambrecht *et al.*, 2004). However, similarly to meat quality traits, lairage time had

299 an effect on the GP of the LT muscle, with muscle lactate and glucose contents and GP
300 values being lower (P = 0.02, P = 0.004 and P < 0.001, respectively) in the LT muscle of
301 pigs kept in lairage overnight (Table 4). Zhen *et al*. (2013) also reported decreased
302 lactate and glucose concentrations and GP value in the LT muscle of pigs as lairage
303 time increased. The GP variation reflects the greater ante-mortem muscle energy
304 exhaustion in the loin muscle of G1 pigs and contributes to explain the variation in pHu
305 in the LT, SM and AD muscles (r = -0.67 and r = - 0.73 for both SM and AD muscles,
306 respectively; P < 0.001 for all muscles), in drip loss (r = 0.57; P < 0.001) and L* value (r
307 = 0.50; P < 0.001) in the LT muscle compared to G2 (Table 5). The correlation between
308 GP of the LT muscle and pHu in the SM muscle is not surprising as these muscles have
309 comparable metabolic characteristics (Laborde *et al.*, 1985; Monin *et al.*, 1987). Indeed,
310 similarly to the LT muscle, in the SM muscle pHu variation follows a curvilinear
311 regression when GP increases (r = - 0.80; P < 0.001; Przybylski *et al*., 1994).

312

313 **Correlations between blood lactate levels and meat quality**

314 Spearman correlations between blood lactate concentration at different sampling points
315 and meat quality traits by lairage event are showed in Table 6. Similarly to Edwards *et*
316 *al*. (2010a), in this study the correlations between blood lactate levels and meat quality
317 traits in the LT muscle were generally low for both lairage groups. The greatest
318 correlation was found between blood lactate level recorded at the end of the resting
319 period when exiting the lairage pen (LA) and the pHu value in the SM and AD muscles (r
320 = 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 1h 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 *post-mortem* acidification rate found in this study confirms what was already reported in previous studies (Hambrecht *et al*., 2005; Edwards *et al*., 2010a). However, the correlations obtained in this study are greater than those reported by Edwards *et al*. (2010a) 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 locomotors 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 *peri-mortem* 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 of the LSA as a tool to predict 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.* Infrared thermography) under more variable preslaughter conditions are needed.

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464 **Table 1.** Spearman correlations between lactate concentrations collected in pigs at four different

465 sampling points[1] 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

466 [1]UN = Unloading (n = 600); LA = End of lairage (n = 583); RE = Restrainer (n = 581); EX:
467 Exsanguination (n = 583).

468 *** $P < 0.001$.

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470

Table 2. Descriptive statistics of blood lactate levels (mM) per lairage group[1] of pigs at four sampling points[2]

	G1				G2			
Sampling point	**n**	**Mean**	**Lower**	**Upper**	**n**	**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

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h

[2]UN= Unloading; LA= End of lairage; RE = Restrainer; EX: Exsanguination

Table 3. Variation of meat quality characteristics in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles of pigs according to the lairage group[1]

Variable	G1			G2			
	n	Mean	SD	n	Mean	SD	*P value*[2]
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

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.

[2]Z -Wilcoxon test.

485 **Table 4.** Variation of lactate content, glucose content and glycolytic potential measured in the

486 *longissimus thoracis* (LT) muscle of pigs from two lairage groups.

	G1	G2	SEM	*P-value*
N	125	150		
Lactate, µmol/g[1]	90.90	95.01	5.16	0.02
Glucose, µmol/g	5.42	6.48	0.30	0.004
GP[2], µmol/g	124.32	134.60	5.96	<0.001

487 [1]All results are presented by µmol/g of meat from the LT muscle at 24h *post-mortem*.

488 [2]GP = Glycolytic potential.

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Table 5. Spearman correlations between glycolytic potential and meat quality characteristics as assessed in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles by lairage group[1,2]

	G1			G2		
Parameters *R*	**GP[3]**	**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***

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.

[2] LT 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).

[3] GP = Glycolytic potential.

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

Table 6. Spearman correlations between blood lactate level at different sampling points[1] on the dressing line and meat quality characteristics as assessed in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles by lairage group[2,3]

	G1				G2			
Parameters *r*	**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, µmol/g [4]	-0.14	-0.33***	-0.26***	-0.16	-0.19*	-0.19*	0.05	0.00
Lactate, µmol/g	0.03	-0.13	-0.16	-0.02	0.07	-0.11	0.06	-0.01
Glucose, µmol/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

[1]UN = Unloading; LA= End of lairage; RE = Restrainer; EX: Exsanguination.

[2]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.

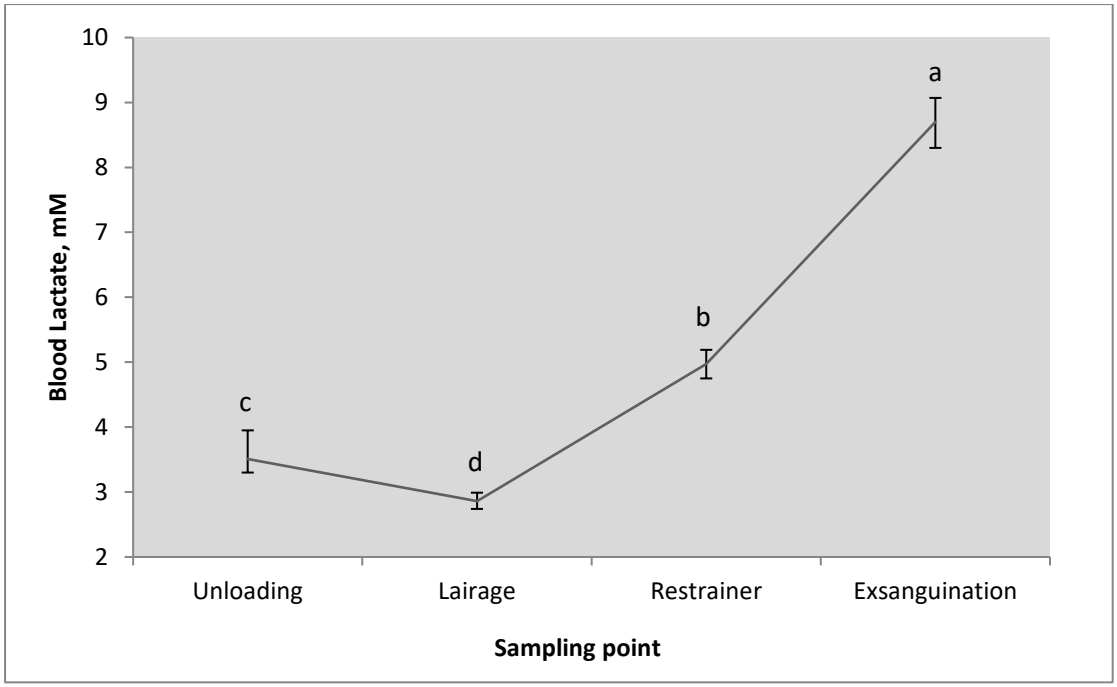
[3] LT muscle (n = 134 for G1 and n = 156 for G2); SM muscle (n = 133 for G1 and n = 155 for G2) AD muscle (n = 135 for G1 and n = 156 for G2).

[4]GP = Glycolytic potential (n = 124 for G1 and n = 148 for G2).

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

FIGURES

Figure 1. Preslaughter variation of blood lactate levels (mM; ± Confidence limits) collected in pigs at four different sampling points* at the slaughter plant.



*Between sampling points, means with a different letter differ significantly ($P < 0.001$).

520 **Figure 2**. Comparison of scores frequency (from 1 to 3)* for floppiness assessed by
521 finger test in the *longissumus thoracis* (LT) muscle of pigs in two lairage groups**.

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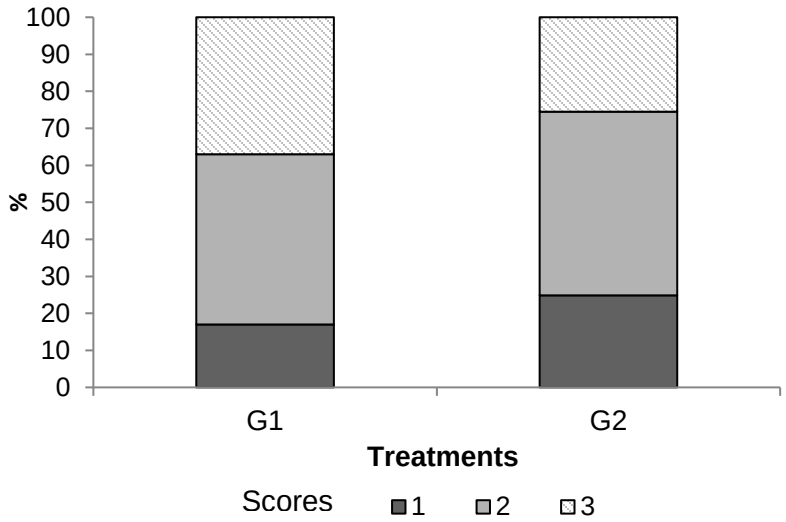

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524 * Floppiness scores: 1 = very soft and watery; 2 = normal and 3 = very firm and dry.

525 **G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.

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