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The application of biosensors for drip loss analysis and glycolytic potential evaluation

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ABSTRACT

The aim of this study was to evaluate the relationship between glucose and lactate measured by biosensors in drip loss (strip method) with muscle glycolytic potential and their compounds. On the samples taken from *Longissimus dorsi* of 24 pigs (pure Neckar hybrid line) the following meat quality traits were determined: pH at 24 h, meat color according to CIE $L^*a^*b^*$ system and drip loss. The highest correlations were found between glucose in drip loss and glycogen ($r = 0.84$) or glycolytic potential ($r = 0.81$) in muscle. A significant positive relationship between lactate measured in muscle by enzymatic method and by biosensor in drip loss was established ($r = 0.76$). Glycogen, glucose, lactate and glycolytic potential with meat quality traits as ultimate pH, lightness, b^* value and drip loss were significantly related. Results of multiple regression between glucose as well as lactate measured in muscle drip loss with muscle glycolytic potential showed the possibility of its prediction ($r = 0.87$ with $P_\alpha \leq 0.001$).

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1. Introduction

Glycogen is the main source of energy for the reconstitution of ATP during anaerobic *post mortem* muscle metabolism when it is converted into lactic acid. The amount of glycogen in muscle depends on genetics and environmental factors that can be divided into the *ante mortem* and *post mortem* (Larzul, Le Roy, Monin, & Sellier, 1998; Meadus & MacInnis, 2000; Rosenvold et al., 2001; Scheffler & Gerrard, 2007). Environmental factors influencing glycogen content and pork quality include· breeding conditions, nutrition, transport conditions, stress, weather conditions and the methods of slaughter. Limiting the stress factors is essential for improving the quality of pork. Muscle glycogen content is key determinant of ultimate pH and other quality traits such as drip loss, color, cooking loss and thus sensory properties of meat (Enfält & Hullberg, 2005; Hamilton, Miller, Ellis, McKeith, & Wilson, 2003; Meadus & MacInnis, 2000; Nanni Costa et al., 2009). The evaluation of glycogen content in live muscle means to operate a muscle biopsy *ante mortem*. In 1985 Monin and Sellier proposed a formula to determine glycogen content in muscle, namely the glycolytic potential (GP) expressed in μmol of lactate, which is the sum of all compounds that are formed in muscle during *post mortem* anaerobic glycolysis as glycogen, glucose-6-phosphate, glucose and lactate. Glycogen is generally analyzed by acid or enzymatic hydrolysis followed by glucose determination (Keeton, Benli, & Claflin, 2009). Maribo, Støier, and Jørgensen (1999) showed that glycolytic potential could be evaluated with the same accuracy both just after slaughter as well as 24 h later. Indeed the impact of glycogen content and its hydrolysate compounds such as glucose on meat quality has been often reported (Enfält & Hullberg, 2005; Hamilton et al., 2003; Meadus & MacInnis, 2000; Nanni Costa et al., 2009). Glucose content was reported to affect the sensory quality, the volatile compounds, the antioxidant activity and the browning level of grilled and fried pork (Buła, Jaworska, & Przybylski, 2015; Meinert, Andersen, Bredie, Bjergegaard, & Aaslyng, 2007; Meinert et al., 2008; Meinert, Schäfer, Bjergegaard, Aaslylg, & Bredie, 2009; Namysław, Buła, Jaworska, Grzywacz, & Przybylski, 2013).

Some studies showed a difference in muscle glucose level after *post mortem* glycolysis which could be attributed to differences in activity of the glycogen debranching enzyme, temperature of muscle or genotype (Meadus & MacInnis, 2000; Scheffler, Kasten, England, Scheffler, & Gerrard, 2014; Ylä-Ajos, Ruusunen, & Puolanne, 2006; Ylä-Ajos et al., 2007). Hamilton et al. (2003) reported a coefficient of variability for free muscle glucose content *post mortem* equal to 74.3% while for GP either measured *in vivo* or *post mortem* the variability is around 22.3% and 24.4% respectively. During glycogenolysis free glucose is liberated by glycogen debranching enzyme that hydrolyses α-1-6 bounds of glucosyl branch (Pösö & Puolanne, 2005). According to Meléndez, Meléndez-Hevia, and Cascante (1997) glycogen molecule is constructed

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from two linear glucose chains named A and B. Each chain contains 13 units of glucose bound together with α-1,4-glycosyl bonds. At the fourth and eighth glucosyl units of the B-chain there is an α-1,6-glycosyl bond initiating a new A-chain, and the total number of chains is $1 + 2^{12}$, *i.e.* about 4100. Information that glycogen molecule contains about 55,000 glucose residues and that some part of glycogen is not degraded (called residual glycogen) allows us to formulate a hypothesis that free glucose could be equal to about 5% of total amount of glycogen. Lundström and Enfält (1997) showed a possible application of glucose determination in meat juice of meat to detect pig carrying RN-gene. Based on the rapid enzymatic hydrolysis of muscle glycogen to glucose a method for early determination of meat ultimate pH was elaborated and patented by Young, Thomson, Merhtens, and Loeffen (2004a). Hargreaves, Barrales, Barrales, Riveros, and Peña (2009) elaborated a rapid and inexpensive method for glycogen determination based on muscle glucose determination. Hamilton et al. (2003) found that the free glucose concentration in the exudate from *Longissimus* muscle was more strongly related to fresh pork quality than glycolytic potential and was quicker and less expensive to measure. Analysis of sarcoplasmic protein in drip loss was also applied by some authors for meat quality evaluation. Bowker, Eastridge, and Solomon (2014) and Żelechowska, Przybylski, Jaworska, and Santé-Lhoutellier (2012) showed that muscle exudates may be a good source of protein markers that are useful in the development of rapid, noninvasive methodologies for predicting pork quality (pH, meat color, drip loss, juiciness) and beef tenderness respectively. Di Luca, Elia, Mullen, and Hamill (2013) stated that muscle exudate provided valuable information about the pathways and processes underlying the *post mortem* ageing period. Therefore we can hypothesize in this study that muscle exudate reflects the changes occurring in meat during aging and its composition. Thus, the objective of this study was to evaluate glucose and lactate measured by a simple method in drip loss as potential markers of muscle glycolytic potential.

2. Materials and methods

2.1. Materials

The research was carried out on 24 pigs (gilts) of pure Neckar line. The Neckar line was produced by PenArLan (France). The Neckar line is characterized by high daily gain and good carcass conformation (especially a large area of the loin "eye"), which enables the production of heavy pigs with high meatiness. Pigs originating from the herds were included in the program of elimination of disadvantageous genes' influence on meat quality (RYR1T and RN-). All animals came from the same farm and were kept under identical environmental conditions. The animals were slaughtered at 100 kg live weight, in the slaughterhouse 50 km far from the farm in accordance with legally binding procedures, according to the following conditions: 2 h resting before slaughter, automatic electric stunning and exsanguinations in the horizontal position, carcass was chilled in fast cooling system.

2.2. Meat quality traits

Samples were taken from *Longissimus dorsi* (LD) muscle behind the last rib (in the lumbar region) within 24 h after slaughter. The following meat quality traits were determined: pH at 24 h *post mortem* with a WTW 330i pH meter (Weilheim, Germany), meat color according to CIE $L^*a^*b^*$ system using CR310 Minolta Chroma Meter with D_{65} light source (Osaka, Japan). The drip loss from muscle tissue was determined 48 h after slaughter according to Honikel (1998). A 50 g sample of meat (10 cm × 5 cm × 1 cm) cut perpendicularly to muscle fibers was taken 24 h *post mortem*, placed in a plastic bag, and kept at the temperature of 4 °C. After 24 h, the sample was removed from the bag, dried on absorbent paper, and reweighed. Amount of drip at 48 h *post mortem* was expressed as a percentage: % drip loss = [(initial weight − final weight)/initial weight] ∗ 100.

2.3. Enzymatic method of glycogen, glucose, glucose-6-phopsphate and lactic acid determination

About 1 g of muscle tissue was homogenized with 10 ml of 0.5 M perchloric acid and homogenate (0.5 ml) was taken for determination of glycogen, glucose and glucose-6-phopsphate according to Dalrymple and Hamm (1973) by enzymatic hydrolysis of glycogen with amyloglucosidase. Lactic acid was determined in the supernatant after centrifuging the homogenate according to Bergmeyer (1974) method. The glycolytic potential (GP) was calculated according to the formula from Monin and Sellier (1985) and expressed as micromoles of lactate equivalent per gram of fresh tissue. All measurements were done in duplicates.

2.4. Determination of glucose and lactic acid by using of biosensors

Twenty four hours after slaughter the samples of LD muscles were placed in a plastic bag and kept at 4 °C for another 24 h for drip loss collection. Drip loss was collected for the glucose and lactate measurements. The determinations of muscle glucose (mmol/l) were made with the Accu-Chek Active® glucometer, that is normally employed for measuring blood glucose in human (Accu-Chek Sensor Comfort®, Roche, Germany). Reactive strips were used for the quantitative determination of glucose with the glucometer in the value range between 0.6 to 33.3 mmol/l. Test results were ready provided 60 s after placing a drop with an approximate volume of 20 μl of drip loss on a reactive strip.

Lactic acid (mmol/l) was measured with the strip method using Accutrend^R Lactate type 3012522 (Roche, Mannheim, Germany). Samples were diluted with distilled water 1:10 to reach a lactate concentration in the following range: 8.7 to 26 mmol/l. The test results also were provided in 60 s after placing a drop with an approximate volume of 20 μl of diluted drip loss on a reactive strip. Then, the results were converted into concentration in undiluted drip loss. All measurements were made in duplicate.

2.5. Statistical analysis

Data were analyzed using the Statistica 10.0 software package (StatSoft, Inc., 2011). For each quantitative variable, the normality was tested using skewness and kurtosis method. The simple Pearson linear correlations and multiple regression between measured traits were calculated as studied sample was homogenous (one breed and sex of

Table 1
Characteristics of meat quality, glycolytic potential and its components in *Longissimus dorsi* muscle and glucose, lactate in drip loss.

Traits	Mean	Min.	Max.	SD
Meat quality traits				
pH_{24}	5.50	5.31	5.68	0.11
Color parameters				
L^*	54.88	49.97	57.29	2.26
a^*	16.23	14.29	17.66	0.88
b^*	5.25	3.14	7.86	0.87
Drip loss (%)	3.51	1.53	6.03	1.03
Muscle longissimus dorsi				
Glycogen[a] (μmol/g)	7.41	2.00	16.62	4.04
Lactate (μmol/g)	106.08	95.27	116.61	5.55
Glycolytic potential (μmol/g)	120.90	102.01	141.82	12.08
drip loss – muscle juice				
Glucose (mmol/l)	6.67	4.50	11.44	1.89
Lactate (mmol/l)	148.96	117.00	179.00	16.29

[a] As sum of glycogen + glucose + glucose-6-phosphate.

Table 2
The correlation coefficients between *Longissimus dorsi* muscle glycolytic potential, glucose and lactate, and glucose and lactate measured in drip loss.

In juice	In muscle	Glycogen (μmol/g)	Lactate (μmol/g)	Glycolytic potential (μmol/g)
Glucose (mmol/l)		0.84*	0.58*	0.81*
Lactate (mmol/l)		0.70*	0.76*	0.81*

* Significant at $P_\alpha < 0.05$.

animals from the same farm, transported and slaughtered in the same time) (Hassler & Thadewald, 2003). The significance of correlations was tested by F-test.

3. Results and discussion

The results characterizing meat quality and carbohydrates in muscle tissue and in muscle juice are presented in Table 1. The mean values of meat quality traits as ultimate pH, color parameters and drip loss were similar to those shown by Żelechowska et al. (2012) and for pigs with rn^+ genotype in the work of Enfält and Hullberg (2005) and Meadus and MacInnis (2000). The results confirmed that the studied pig population was free from RN^- gene which is known to increase the glycogen level in muscle and to decrease ultimate pH (Enfält & Hullberg, 2005; Meadus & MacInnis, 2000; Scheffler & Gerrard, 2007). The glycolytic potential was similar to the values reported by Żelechowska et al. (2012) in Neckar line pigs with normal meat, or by Traore et al. (2012) in Galia line or by Meadus and MacInnis (2000) in rn^+ genotype pigs. Similar values were also found in Landrace × Yorkshire crossbreed pigs with $DECR1^DDECR1^D$ genotype by Kamiński et al. (2010). On the contrary, Italian Large White muscle pigs (Nanni Costa et al. (2009), Redone line pigs (Traore et al. (2012) showed lower glycolytic potential. As expected, higher glycolytic potential was reported in pig with RN^- genotypes (Meadus & MacInnis, 2000) and in Neckar line pigs with acid meat (Żelechowska et al., 2012). Values with significant variation (lower and higher) depending on the CAST genotype were showed by Koćwin-Podsiadła, Kurył, Krzęcio, Zybert, and Przybylski (2003) in crossbreed pigs of Polish Large White × Polish Landrace × Hampshire × Pietrain breeds.

The lactate was the main compound (70–88%) of GP measured 24 h *post mortem* which was consistent with the results of many authors (Bidner, Ellis, Witte, Carr, & McKeith, 2004; Edwards et al., 2010; Fernandez, Neyraud, Astruc, & Sante, 2002; Maribo et al., 1999). The measurement taken 45 min after slaughter showed that the amount of lactate was already about 50% of the maximum produced *post mortem*. Nevertheless the method developed by Monin and Sellier (1985) enables measurement of glycolytic potential at any time after slaughter. Values of PG measured before slaughter in biopsy samples were higher by about 50 μmol/g of fresh tissue (Fernandez et al., 2002; Hamilton et al., 2003; Koćwin-Podsiadła, Przybylski, Fernandez, & Monin, 2001). The studies by Maribo et al. (1999) showed that the values of PG measured 4 min and 30 h after slaughter were similar. Four minutes after slaughter glucose, G-6-P, glycogen and lactate account for about 2.4%, 11.6%, 65.8% and 20.2% respectively. At 30 h *post mortem*, glucose, G-6-P, glycogen and lactate account for 7.6%, 10%, 1.9% and 70.5% respectively. The glucose concentration in muscle juice (drip loss) was comprised between 6 and 7 mmol/l. It was similar to the results reported by Hamilton et al. (2003) in pork exudate and by Young et al. (2004a) in beef measured by glucometer sensor after glycogen hydrolysis with amyloglucosidase. Those values were also similar to the blood glucose level in pigs as shown in the study by Choe et al. (2009). The concentration of lactate was closed to 150 mmol/l. To the best of our knowledge, this is the first study reporting the assessment of lactate in muscle juice. Edwards et al. (2010) reported a lactate concentration in exsanguination blood in pigs ranged from 4 to 19.7 mM and suggested that such measurement was predictive of the rate of early *post mortem* metabolism. These authors demonstrated a significant relationship between blood lactate concentration and pH measured 60 min. after slaughter ($r = -0.32$) and drip loss ($r = 0.22$). Table 2 showed the correlation coefficients obtained between glycogen, lactate and GP measured in muscle tissue, and glucose and lactate assessed in drip loss. The correlation coefficient was comprised between 0.58 and 0.84. For lactate, a correlation coefficient of 0.76 was found between the enzymatic method and the strip method. The highest correlations were found between glucose in juice and glycogen ($r = 0.84$) or GP ($r = 0.81$) in muscle. They were all statistically significant. These results are in accordance with those reported by Enfält and Hullberg (2005) who highlighted that the carbohydrates with 6 carbons in drip loss reflected the glycogen content in meat. A correlation coefficient between free glucose measured in muscle juice and in live-animal or *post mortem* glycolytic potential measured in muscle equal to 0.51 and 0.7 respectively was pointed out by Hamilton et al. (2003). This result suggests that the measurement of glucose originating from muscle e.g. in meat juice could be a good estimator of the quantity of muscle glycogen. One explanation about this link between drip loss and meat composition is that in living muscle the water is held within the myofibrils in the spaces between the thick and thin filaments, and rigor onset results in a migration of water and some soluble proteins outside the cell (Offer et al., 1989). During the conversion of muscle to meet the shrinkage of myofibrils and as its consequence reducing the space available to hold the myowater, disintegration of cellular membranes during the development of *rigor mortis* as well as formation of drip channels (gaps) gives possibility to the water leakage together with solubilized components (Offer et al., 1989; Pearce, Rosenvold, Andersen, & Hopkins, 2011). For this reason muscle juice obtained during aging reflects meat composition and provided valuable information about the pathways and processes occurring in this tissue after slaughter (Di Luca et al., 2013).

Muscle glucose measurement for evaluation of glycogen level in muscle is applied in methods based on acid or enzymatic hydrolysis of glycogen (Keeton et al., 2009). Recently Hargreaves et al. (2009) elaborated a practical, rapid and inexpensive method for muscle glycogen determination based on acid hydrolysis and then glucose determination using a domestic glucometer for human blood glucose level control. Free glucose measured in drip loss can reflect the total amount of glycogen due to the fact that only phosphorylated glucose can be included to anaerobic glycolysis occurred in muscle *post mortem*. The proposed strip method of drip loss glucose assessment with the glucometer is feasible, convenient. The glucose evaluation can be carried out easily with low cost. Studies of Copenhafer, Richert, Schinckel, Grant, and Gerrard (2006) and Maribo et al. (1999) demonstrated that the amount of free glucose was equal 3–4% of the glycolytic potential. Probably it depends to activity of glycogen debranching enzyme. Ylä-Ajos et al. (2006, 2007)

Table 3
The correlations coefficient between glycolytic potential and its components, glucose and lactate in drip loss with meat quality traits.

	pH_{24}	L	a	b	Drip loss (%)
Glycogen (μmol/g)	−0.80*	0.82*	−0.26	0.48*	0.52*
Lactate (μmol/g)	−0.45*	0.38	−0.06	0.35	0.08
Glycolytic potential (μmol/g)	−0.74*	0.72*	−0.20	0.48*	0.39
Glucose (mmol/l)	−0.71*	0.81*	−0.10	0.54*	0.55*
Lactate (mmol/l)	−0.71*	0.70*	−0.07	0.48*	0.21

* Significant at $P_\alpha < 0.05$.

Table 4
The multiple regression between muscle glycolytic potential and glucose and lactate measured in drip loss.

The regression equation		$P_\alpha \leq$	Total R for regression
Intercept	49.45	0.01	
Glucose (G)	3.13	0.01	$R = 0.87\ F_{(2.20)} = 30.16\ P_\alpha \leq 0.001$
Lactate (L)	0.34	0.01	

showed that the activity of glycogen debranching enzyme depends on temperature, animal species, muscle and RN genotype. Hamilton et al. (2003) concluded that free glucose concentration in the exudate from longissimus muscle was more strongly related to the fresh pork quality (L^* value, drip loss, ultimate pH) than glycolytic potential and was quicker and less expensive to measure. Lundström and Enfält (1997) showed that the measurement of free glucose in meat juice can be a good and rapid prediction of pigs carrying of RN^- gene (*i.e.* large quantity of glycogen in muscle).

Table 3 shows the correlation between glycogen, glucose, lactate and glycolytic potential and meat quality traits such as ultimate pH, lightness, b^* value and drip loss. Similar relationships have been reported by several authors (Hamilton et al., 2003; Meadus & MacInnis, 2000; Nanni Costa et al., 2009; Young, West, Hart, & van Otterdijk, 2004b). Hamilton et al. (2003) reported a significant relationship between free glucose and L^* value, drip loss, ultimate pH equal to 0.52, 0.55, −0.63 respectively. Meadus and MacInnis (2000) pointed out positive correlation coefficients between glycogen, lactate and glucose with L^* value (0.31–0.38) and negative ones with ultimate pH (from −0.40 to −0.68). These results confirm significant effect of glycogen on meat quality traits as pH, meat color and drip loss. In order to express the glycolytic potential as a function of the variables measured in drip loss, a multiple regression was conducted. The equation is given below ($y = b_0 + b_{1\times 1} + b_{2\times 2}$):

$$\text{Glycolytic Potential} = 49.45 + 3.13 * \text{Glucose drip} + 0.34 * \text{Lactate drip}$$

The R (coefficient of correlation) value was 0.87 with $P_\alpha \leq 0.001$ (Table 4).

These results of multiple regression between glucose as well as lactate measured in muscle drip loss with muscle glycolytic potential showed the possibility of its prediction.

4. Conclusion

The study showed existence of significant relationship between glucose and lactate measured in drip loss with muscle glycogen, lactate, glycolytic potential and meat quality traits. This study clearly showed that simple measurement of glucose and lactate by applications of biosensors (with the strip method in exudate) can be good predictors of glycolytic potential, which is known as a technological meat quality parameter. The availability and simplicity of glucose and lactate evaluation in drip loss with strips may show the possibility of its practical applications for glycolytic potential evaluation, but needs confirmations in additional experiment.

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