Classification accuracy of different pork quality evaluation methods in assessment of meat with lowered drip loss

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ABSTRACT: This study compared the diagnostic value of pork quality evaluation methods using different pH threshold values and time-points with muscle metabolites concentration threshold values measured 45 min. post mortem in assessment of meat with lowered drip loss. Samples of 100 longissimus dorsi (LD) (Landrace × Yorkshire) × Duroc fatteners were examined after slaughter for following parameters: muscle acidity in 35 min, 2 h, 3 h, 24 h and 48 h (pH_p, pH₂, pH₂, and pH₄), colour lightness (L^{*}, a^{*}, b^{*}), meat yield after curing and thermal processing in 72 °C (technological yield), water-holding capacity (WHC) and drip loss in 48, 96 and 144 h (DL₄₈, DL₉₉, DL₁₄). To verify the accuracy of analysed methods two groups were distinguished according to DL₄₉, e.g. Low DL (DL₄₈ ≤4%) and High DL (DL₄₈ >4%). In High DL pH₁ to pH₄₈ were statistically lower while L^{*}, WHC, DL₄₉, DL₉₉, DL₁₄₄ were statistically higher (P≤0.05). On the basis of pH-dependent methods classification to RFN (red, firm, normal), PSE (pale, soft, exudative), DFD (dark, firm, dry) and AM (acid meat) was performed and then the percentage share of Low DL and High DL among meat classified as RFN was evaluated. Despite most samples were classified as RFN Low DL share among them did not exceed 50%. If meat sample shows metabolites concentration below threshold value and was assigned to Low DL (or was assigned to High DL above threshold value) it was regarded as correctly classified. The most promising cut-off point (correct classification of 73%) was 45 µmol both for glycogen and lactate.

Precisão da classificação de métodos de avaliação da qualidade da carne suína para menor perda durante o gotejamento

RESUMO: Neste estudo foram analisadas 100 amostras de longissimus dorsi (LD) de suinos Landrace, Yorkshire ($L \times Y$) × Duroc (D). Dois grupos foram distinguidos de acordo com a perda por gotejamento medida 48 horas após o abate, por ex. DL baixo ($DL_{48} \leq 9\%$) e DL alto ($DL_{48} \geq 4\%$). Em DL alto maior leveza (L^*), capacidade de retenção de água (WHC), perda por gotejamento em 48 (DL_{48}), 96 (DL_{90}), 144 (DL_{144}) e menor acidez muscular de 35 min. a 48 horas post mortem (pH_1 a pH_{48}) foram anotados ($P \leq 0.01$). A baixa participação de DL dentro das amostras classificadas como RFN (vermelha, firme, normal) com base em vários métodos de avaliação da qualidade da carne suína usando diferentes pontos de tempo de pH e valores de limiar não excederam 50%. A tentativa de uso de várias concentrações de metabólitos musculares mediu 45 min. post mortem (glicogênio, lactato e suas combinações) como valores limiares na avaliação de DL baixo e DL alto foi então realizado. Os pontos de corte mais promissores (45 µmol por g de tecido muscular tanto para glicogênio quanto para lactato) permitiram classificar corretamente 83.82% de DL baixo e 50% de DL alto respectivamente abaixo e acima deles. **Palavras-chave**: perda por gotejamento, glicogênio, lactato, pH, qualidade da carne suína.

INTRODUCTION

According to JOO et al. (2013) modern consumers demands for high meat quality still increase so this needs should be fulfilled with consistent production of tasty, safe and healthy products that will ensure continuous consumption in future. Nowadays both in Europe and in the US the majority of purchased meat (that is still rising) is unprocessed, e.g. fresh (DANIEL et al., 2011). Undoubtedly one of the most important attributes of fresh pork besides its colour, texture and amount of fat (intramuscular/ intermuscular/subcutaneous) is drip loss that occurs during muscle to meat conversion (JOO et al., 2013). Because of consumer demands it should be minimized to reduce negative impact to appearance and sensory quality of meat that it generates (TROY & KERRY, 2010). Decrease in exudation is also of great interest for the retail market due to display time elongation of case-ready meat products (OTTO

Received 09.22.20 Approved 12.04.20 Returned by the author 03.19.21 CR-2020-0885.R1 et al., 2007). Excessive drip loss in fresh pork also lowers technological quality, i.e. processing yield that generates considerable financial losses borne by the processors (HUFF-LONERGAN & LONERGAN, 2007). According to FISCHER (2007) in Germany per 1% of excessive drip loss in pork loin financial losses of 19.8 million \notin is generated annually and according to KNOX et al. (2008) the U.S. pork industry loses more than \$100 million annually due to quality defects.

In view of the above the correct prediction of fresh pork with low technological quality is of crucial importance; and therefore, different approaches was established to evaluate drip loss worldwide. Most of them cannot be used directly in the abattoir and are executed after 24 hours post mortem so their usefulness in sorting of carcasses is low (PRANGE et al., 1977; KAUFFMAN, 1993; CHRISTENSEN, 2003; CORREA, 2007; KAPPER et al., 2014). Significant differences in results between these methods are also observed due to various procedures used (FILHO et al., 2017). It is also common to classify pork into quality classes (PSE - pale, soft, exudative; RFN - red, firm, nonexudative; DFD - dark, firm, dry and AM - acid meat) on the basis of several criteria measured up to 24 hours after slaughter but both selection of these criteria and their threshold values differ amongst most countries (KAUFFMAN et al., 1993; RAUW et al., 2003; KOĆWIN-PODSIADŁA et al., 2006; FAUCITANO et al., 2010; POSPIECH et al., 2011; PRZYBYLSKI et al., 2012; TARCZYŃSKI et al., 2018; KIM et al., 2016). Therefore, the frequency distribution of pork quality classes could vary which was recently confirmed by CAZADEY et al. (2016). Anyway occurrence of pork with high drip loss is still a common phenomena (BARBUT et al., 2008; FAUCITANO et al., 2010; CAZADEY et al., 2016).

Muscle acidity (pH) decline and extent is generally accepted to be one of the most important factors connected with drip loss. Early pH measurements of pork could be used for carcass sorting but their application in abattoirs is rather limited (KOĆWIN-PODSIADŁA et al., 2006). Also, ultimate pH (pH_a) due to relatively late time of execution and probability of its elongation could also decrease its usefulness (TARCZYŃSKI et al., 2018). Conversely, muscle glycogen being converted post mortem to lactate and H⁺ ions is also responsible for proper muscle acidification and highly determine pH decrease rate and extent (SCHEFFLER et al., 2013). This complex biochemical process known as glycogenolysis is the most crucial factor in muscle to meat conversion in which according to POSO & PUOLANNE (2005) the carbohydrate metabolism is of the highest importance. However, there is still lack of knowledge about the potential usefulness of muscle metabolites concentration measured after slaughter for diagnostic purposes.

This study compared the diagnostic value of pork quality evaluation methods using different pH time-points and threshold values with different muscle metabolites concentration threshold values measured in pre-rigor state (glycogen, lactate and their combinations) in sorting of carcasses with lowered drip loss.

MATERIALS AND METHODS

Animals, slaughter and carcass treatment

The investigation was carried out in autumn on 100 (Landrace × Yorkshire) × Duroc fatteners (50 gilts and 50 barrows) originated from one producer (Mazowsze district, Poland). At the farm, animals were kept under the same environmental conditions (concrete floor) and fed complete diet according to age with ad libitum water accessibility. After reaching a body weight of about 105 kg fatteners were loaded (no electrical pods were used) in small groups by qualified personnel to transport vehicles. The transport was performed at night (approximately for 280 km). After unloading in meat plant animals were moved to large pens for 4 hours rest with easy access to fresh water and afterwards moved to stunning area by trained personnel using paddles and hydraulically powered restraint equipment. Fatteners were stunned using automatic electrical stunner (MIDAS, Stork RMS, the Netherlands and INARCO constant voltage system) and exsanguinated horizontally in accordance with the meat plant technology. Lean meat content was determined 35 min post mortem by ULTRA-FOM 300 (SFK-Technology) and hot carcass weight (HCW) was measured immediately afterwards (accuracy up to 0.1 kg). Subsequently, carcasses were chilled in three-phase chilling tunnel (-10 °C for 15 min., -15 °C for 25 min. and -5 °C for 40 min. with air velocity of 3 m/s) and stored at 4 °C up to 24 h after slaughter.

Meat quality attributes

All of examined meat quality attributes were measured directly in hanging carcasses in the longissimus lumborum muscle (LL) behind the last rib (from 35 min. to 24 h *post mortem*) or in meat samples taken at last rib and 1st lumbar vertebra (after 24 hour after slaughter). Each of muscle sample was separated from the bone, external fat and epimysium and then stored in plastic bags at 0-4 °C. Acidity of muscle tissue (pH) was measured 35 min., 2 h, 3 h, 24 h and 48 h (pH₁, pH₂, pH₃, pH₂₄ and pH₄₈ respectively) using a pistol pH-meter MASTER (Draminski, Olsztyn, Poland) with temperature compensation. Water-holding capacity (WHC) was measured after 24 hours by the filter paper press method (Whatman 4 filter paper) according to GRAU & HAMM method (1953) modified by POHJA & NINIVAARA (1957). Drip loss was assessed 48 h, 96 h and 144 h (DL₄₈, DL_{96} , DL_{144} respectively) according to PRANGE et al. (1977). Meat color lightness (L*) and its components (a* - red and b* - yellow) was measured 24 h with a Minolta Chroma Meter (model CR 310, Minolta, Osaka, Japan) using D65 illuminant and 50 mm orifice. Meat yield after curing and thermal processing in 72 °C was expressed by the TY (technological yield) indicator according to NAVEAU et al. (1985).

Muscle metabolites concentration

Immediately after pH_1 measurement LL muscle samples (1 g per carcass) were taken and immersed (up to 45 min. *post mortem*) into tubes with 10 ml of 0.5M HCLO₄ and then homogenized to inhibit glycogen changes in muscles. Samples were stored at -20 °C for 3 weeks. Glycogen

concentration was determined by enzymatic method according to DALRYMPLE & HAMM (1973) using amyloglucosidase derived from the yeast *Aspergillus niger*. Lactate concentration was determined according to BERGMEYER (1978) using lactate dehydrogenase. The glycolytic potential (GP) was calculated as the sum of: 2 [glycogen] + [lactate] according to simplified formula of MONIN & SELLIER (1985) and expressed as µmol of lactic acid equivalent per g of fresh muscle tissue.

Polymorphism of RYR1 gene

The genomic DNA was isolated from white blood cells according to KAWASAKI (1990). *RYR1* C1843T polymorphic site was analysed with DNA test using the PCR/RFLP method according to FUJII et al., (1991). No *RYR1*^T*RYR1*^T genotypes were diagnosed.

Meat quality evaluation methods

In this study the comparison of pork quality evaluation methods using different pH threshold values and time-points with muscle metabolites concentration threshold values measured 45 min. *post mortem* in assessment of meat with lowered drip loss was performed. In table 1 pH-dependent methods were shown (different pH threshold values and time-points) while muscle metabolites

Table 1 - Pork quality evaluation methods using different pH time-points and threshold values.

Meat quality class	Evaluation methods						Time- points	
	RAUW et al., 2003 pHI	PRZYBYLSKI et al., 2012 pHII	POSPIECH et al., 2011 pHIII	KOĆWIN- PODSIADŁA, 2006 pHIV	PIC, 2003 pHV	PIC, 2003 pHV ^{m1}	PIC, 2003 pHV ^{m2}	
RFN	≥6.00 * 5.30–6.20	≥ 6.0 ≥ 6.0 $\geq 5.5 - < 5.8$	>5.8 * 5.5–6.0	≥6.0 * 5.5–6.0	6.3- 6.7 * 5.7-	6.3– 6.7 * 5.5–	* * 5.7–6.1	
PSE	<6.20 * 5.60–5.99	<6.0 *	≤5.8 * ≤5.5	<6.0 * <5.5	6.1 <5.8 * <5.5	6.1 <5.8 * <5.5	* * *	$\begin{array}{c} pH_1 \\ pH_3 \end{array}$
DFD	≥6.00 * >6.20	* * *	>6.0 * >6.0	$\geq 6.0 \\ * \geq 6.0$	>6.7 * >6.1	>6.7 * >6.1	* * *	pH ₂₄
AM	* * <5.30	$\geq 6.0 \\ \geq 6.0 \\ < 5.5$	* * *	$\geq 6.0 \\ * \\ < 5.5$	* *	* *	* *	

* - no threshold value assigned; m - modified.

concentration threshold values measured 45 min. post mortem were presented in table 2. To verify the accuracy of examined evaluation methods two groups regarding drip loss measured 48 hour post *mortem* were distinguished, e.g. Low DL - $DL_{49} \leq 4\%$ and High DL - $DL_{48} \ge 4\%$ and characterized by quality attributes described in previous section. In case of pHdependent methods the classification to RFN, PSE, DFD and AM was performed. Taking into account that sample classified as RFN may not necessarily stand for lowered drip loss the percentage share of Low DL and High DL within meat classified as RFN was carried out. If pH of certain sample did not fulfil threshold values or certain class was not assessable by examined method it was regarded as unclassified. The attempt of assessment of meat samples with lowered drip loss using muscle metabolites, e.g. glycogen and lactate concentration was performed on the basis of their strict threshold values (cut-off points). If meat sample shows muscle metabolites concentration below certain threshold value and simultaneously was assigned to Low DL it was regarded as correctly classified. If muscle metabolites concentration was above certain threshold value and simultaneously assigned to High DL it was also regarded as correctly classified. The aforementioned attempt allowed to eliminate possibility of improper classification of meat samples to High DL below respective threshold values and to Low DL above them. Muscle metabolites threshold levels, e.g. glycogen and lactate were examined solely and in combinations.

Statistical analysis

Data were analysed by one-way analysis of variance using non-orthogonal contrast in STATISTICA 13.1 (StatSoft, Tulsa, OK, USA). The model was expressed as follows: $y_i = \mu + a_i + e_i (y_i$ - measured i_{th} trait, μ - overall population mean, a_i analysed factor effect of i_{th} trait, e_i - random error). The significance of differences between means was calculated using Tukey's test.

RESULTS

Carcass characteristics and meat quality attributes

All research material was characterized by lean meat content of $58.4\pm2.32\%$ with hot carcass weight of 86.2 ± 3.27 kg (Table 3). No statistical differences in HCW, lean meat content, a^* , b^* , TY and glycogen concentration were reported between Low DL and High DL groups. Statistically lower (P \leq 0.05) pH₁ to pH₄₈, glycolytic potential and lactate concentration and higher lightness (L^{*}), WHC, DL₄₈, DL₉₆ and DL₁₄₄, values were noted in High DL (Table 3).

Classification to meat quality classes and accuracy in drip loss evaluation

On the basis of pH-dependent pork quality evaluation methods high percentage of RFN meat, e.g. 100% in pHI, 92% in pHII, 98% in pHIII and pHIV and 78% in pHV^{m1} was classified (Table 4). The threshold value for pH₂₄ of abovementioned methods was 5.5. The main difference to these results was only 36% of meat samples classified as RFN with pHV in which pH_{24} threshold was higher (5.7) and 47% in pHV^{m2} in which pH, was excluded (Table 4). Although, most of meat samples were classified as RFN with pHI, pHII, pHIII, pHIV and pHV^{m1} very limited share of Low DL were noted within them (33%, 35.87%, 32.65%, 32.65% and 32.05% respectively). More accurate methods in assessment of pork with lowered drip loss were pHV and pHV^{m2} (44.44% and 46.68% of Low DL share respectively). Pork samples correctly classified as Low DL below analysed muscle metabolites threshold values ranged from 25% in G35 to 56.25% in G45 and from 40.63 in L35 to 90.63% in L50 (Table 1). Irreversibly, the correctly classified High DL samples above examined cut-off points decreased from 89.71% in G45 to 48.82% in G35 and from 77.94% in L50 to 19.12% in L35. The highest correctly classified Low DL share below and High DL above combined glycogen and lactate threshold values was 83.82%

Table 2 - Pork quality evaluation methods using muscle metabolites concentration threshold values measured 45 min. post mortem.

	Muscle metabolites concentration threshold values measured 45 min. post mortem [µmol per g of muscle tissue)						
Glycogen		Lactate	Glycogen/Lactate				
35 (G35)		35 (L35)	40/40 (G40/L40)				
40 (G40)		40 (L40)	40/45 (G40/L45)				
45 (G45)		45 (L45)	45/40 (G45/L45)				
		50 (L50)	45/45 (G45/L50)				

Traits		Groups		Р
	Low DL	High DL	Total	
HCW [kg]	85.8±3.34	86.9±3.90	86.2±3.72	0.32
Lean meat content [%]	58.1±2.05	58.5±2.44	58.4±2.32	0.76
pH ₁	6.6±0.11 ^a	6.5±0.15 ^b	6.6±0.15	0.01
pH ₂	6.5 ± 0.1^{a}	$6.4{\pm}0.19^{b}$	6.5±0.18	0.03
pH ₃	$6.4{\pm}0.1^{a}$	6.2 ± 0.20^{b}	6.3±0.19	0.05
pH ₂₄	5.7±0.1ª	5.6±0.09 ^b	5.7±0.11	< 0.00
pH ₄₈	5.5±0.1ª	5.5 ± 0.09^{b}	5.5±0.10	< 0.00
L*	53.5±2.79 ^b	$55.0{\pm}2.6^{a}$	54.5±2.75	0.04
a [*]	13.8±1.45	14.2 ± 1.20	14.0±1.29	0.18
b*	4.7±1.29	5.1±1.20	4.5±1.24	0.75
TY [%]	100.8 ± 7.21	101.4±7.28	101.2±7.23	0.31
WHC [cm ²]	4.5±1.13 ^b	5.4±1.1 ^a	5.1±1.22	0.02
DL ₄₈ [%]	2.8 ± 0.88^{b}	6.7±2.3ª	5.4±2.69	< 0.00
DL ₉₆ [%]	$6.0{\pm}1.42^{b}$	9.8±2.3ª	8.5±2.74	< 0.00
DL ₁₄₄ [%]	8.7 ± 2.39^{b}	12.0±2.6 ^a	10.9 ± 3.02	< 0.00
Glycolytic potential [µmol/g]	126.5±25.40 ^b	136.4±18.00 ^a	133.3±20.99	0.04
Glycogen concentration [µmol/g]	44.2±11.50	47.0±9.99	46.1±10.51	0.16
Lactate concentration [µmol/g]	38.0 ± 8.57^{b}	42.4 ± 9.00^{a}	41.0±9.05	0.04

Table 3 - Carcass characteristics, meat quality attributes and muscle metabolites measured 45 min post mortem.

Values were presented as means and standard deviations (±SD); Means followed by different letters on the same line are significantly different by Tukey's test at 5% probability level.

and 50% respectively (G45/L45) that resulted in 73% of overall correct classification (Table 5).

DISCUSSION

Carcass characteristics, meat quality traits and muscle metabolites concentration

No statistical differences noted in lean meat content and hot carcass weight between Low

DL and High DL were in accordance with study of TRAORE et al. (2012). It has to be stated though that cited authors found experimental group with $DL_{48}>4\%$ approximately 8 kg lower in HCW besides similar DL_{48} mean value (7.72%) to our findings for High DL. In study of RYBARCZYK et al. (2018) similar pH measured 35 min (6.58±0.15) and 24 hours (5.65±0.16) after slaughter were reported in (Landrace × Yorkshire) × DanAvl Duroc. However

Table 4 - Classification to meat quality classes on the basis of different pH time-points and threshold values along with Low DL share within meat classified as RFN.

Meat quality classes	Evaluation methods						
	pHI	pHII	pHII	pHIV	pHV	pHV^{m1}	pHV ^{m2}
RFN [%] Low DL [%]	100 (33)	92 (35.87)	98 (32.65)	98 (32.65)	36 (44.44)	78 (32.05)	47 (46.68)
PSE [%]	0	0	0	0	0	0	*
DFD [%]	0	0	1	1	0	0	*
AM [%]	0	1	*	1	*	*	*
Unclassified [%]	0	7	1	0	64	22	53

* - no threshold values assigned; RFN - red, firm, non-exudative, PSE - pale, soft, exudative; DFD - dark, firm, dry, AM – acid meat; Low DL - drip loss measured 48 hours *post mortem* $\leq 4\%$.

Muscle metabolites threshold values	Correct classification to Low DL below threshold value	Correct classification to High DL above threshold value	Correct classification (overall)
G35	25.00%	89.71%	69.00%
G40	43.75%	76.47%	66.00%
G45	56.25%	48.82%	58.00%
L35	40.63%	77.94%	65.00%
L40	40.63%	61.76%	60.00%
L45	84.38%	41.18%	55.00%
L50	90.63%	19.12%	42.00%
G40/L45	37.50%	92.50%	75%
G40/L50	40.63%	83.82%	70%
G45/L45	83.82%	50.00%	73%
G45/L50	67.65%	53.13%	43%

Table 5 - Correct classification to Low DL and High DL on the basis of different threshold level values of glycogen,	lactate and their
combinations.	

Low DL – drip loss measured 48 hours *post mortem* \leq 4%; High DL – drip loss measured 48 hours *post mortem* >4%; G –glycogen concentration measured 45 min *post mortem* expressed in µmol per g of muscle tissue, L – lactate concentration measured 45 min *post mortem* expressed in µmol per g of muscle tissue.

cited authors noted lower pH₃ (5.91±0.19) and higher pH_{48} (5.72±0.20) and pH_{96} (5.68±0.14). Lower pH_{11} (5.86 ± 0.18) and pH₂₄ (5.51 ± 0.01) were also noted by ZENG et al. (2019) in (Landrace × Yorkshire) × Duroc. Abovementioned could possibly occur due to differences in carcass cooling regime (in present survey three-phase chilling tunnel was used). Contrary to our findings KAPPER et al. (2014) reported no statistical differences in pH, between experimental groups differentiated by DL₄₈ level. Cited authors found instead statistically lower pH₃ and pH_{24} (P ≤ 0.05) in meat samples with DL₄₈ above 10%. In our study; however, such high drip loss occurrence was very limited. TRAORE et al. (2012) found no differences in pH1, pH3 and pH24 between pork samples differentiated by DL48 level in Naïma × P76 fatteners. In contrast, KIM et al. (2016) reported higher DL48 (P≤0.05) in Berkshire fatteners if pH₁ was below 6.3 and that higher DL_{48} was strictly associated with lower pH24. The aforementioned could possibly indicate that pH use in pork quality evaluation could be additionally limited due to different results depending on pig breed. Slightly higher values of L* (55.24±2.78) and notably lower a* (5.10±1.06) and higher b* (13.34±0.60) were reported by RYBARCZYK et al. (2018). ZENG et al. (2019) found lower L* (50.98±0.64) and a* (6.51±0.58) with similar b* (4.90±0.25). TRAORE et al. (2012) found rather weak statistical differences (P < 0.0663) in meat lightness between different DL₄₀

ranges among Naïma×P76 fatteners. Similarly to our findings no statistical differences in a* were found. In study of RYBARCZYK et al. (2018) and ZENG et al. (2019) lower values of DL_{48} were noted (2.19±0.99% and 2.83±0.19% respectively). However in present survey DL_{48} value was 5.44±2.69% which confirms that variability in pork drip loss is rather high.

Evaluation methods using different pH time-points and threshold values

According to BOLER et al. (2010) one of the main goals of pork industry is to improve, predict and reduce variation in pork quality. Due to fact, that several quality attributes of fresh pork including colour, WHC and drip loss are determined by muscle pH fall and extent its measurement at different time-points after slaughter is widely used for quality evaluation. SCHAFER et al. (2002) reported that variation in pH measured up to 24 hours after slaughter determine 90% of variation in DL₄₈. In study of FISHER (2007) 75% of carcasses could be correctly classified regarding drip loss amount by pH, threshold value of 6.2. However, in present study the highest share of Low DL within meat samples classified as RFN on the basis of remain pH-dependent methods (including pH₁ and pH₂) did not exceed 50% (Table 4). This could possibly indicate that measurement of pH fall and extent only is not sufficient for proper quality evaluation due to complexity of post mortem muscle metabolism. Additionally BOLER et al. (2010)

suggested that pH_1 and pH_3 are of low predictive value if analysed population is characterised by small variation. As reported by cited authors low values of pH_1 and pH_3 may also not always correspond to quality deterioration; therefore, pH_{24} is much better parameter in prediction of pork quality. This is partly in accordance with our findings: both Low DL and High DL experimental groups were characterised by rather high pH_1 , pH_2 and pH_3 values; however, pH_{24} solely (pHV^{m2}) allows to differentiate 32% of RFN meat among with only 46.88% were correctly classified to Low DL (Table 4). Low predictive value of pH_1 and pH_{24} in pork ultimate quality prediction was also noted by TOMOVIC et al. (2014).

Evaluation methods using muscle metabolites levels measured 45 min. post mortem

Although, muscle metabolites determined pork quality concentration highly attributes their whole potential in quality evaluation is not yet fully known. MATARNEH et al. (2015) show that variation in ultimate pH could be explained by differences in buffering capacity of muscle and indicated that additional 10 µmol of lactate per g of muscle tissue produced during post mortem glycolysis reduce pH of muscle by 0.2 units. KOĆWIN-PODSIADŁA et al. (2009) noted that the increase in glycogen concentration measured 45 min. after slaughter by 10 µmol per g of muscle tissue resulted in considerable increase in DL_{48} and DL_{144} (by 0.9% and 1.6% respectively). Although, ZYBERT et al. (2020) reported no statistical differences in DL_{48} within three ranges of glycogen concentration measured 45 min after slaughter (<35, 35-55 and $>55 \mu$ mol per g of muscle tissue). In present study the highest share of correctly classified meat samples (to Low DL or High DL) on the basis of glycogen concentration was 69% (G35). This substantial value arises from meat samples correctly classified to High DL (89.71%) but not to Low DL (37.50%). Higher correct classifications of meat samples to Low DL were noted in G40 and G45 (43.75% and 56.25%) respectively). Lactate concentration threshold values of 35 (L35) and 40 (L40) µmol per g of muscle tissue allowed to correctly classify meat samples in 65% and 60% respectively. Similarly to G35 these values arise from high share of correctly classified High DL (77.94% and 61.76% respectively). With 45 (L45) and 50 (L50) umol of lactate per g of muscle tissue higher correctly classified shares of Low DL were achieved (respectively 84.38% and 90.63%). Taking that into account it can be presumed that glycogen level is more useful in correct classification to High

DL while lactate concentration to Low DL. However, full efficiency is not ensured.

The most useful combination of glycogen and lactate concentration threshold values (G45/ L45) allows to correctly classify 83.82% of analysed meat samples to Low DL below and 50% to High DL above them with overall correct classification of 73% (c.a. 25 p.p. more than in pHV and pHV^{m2}). Similarly SHAFER et al. (2002) reported that muscle metabolites did not explain entire variance in drip loss (glycogen concentration determine it in 73% and lactate concentration in 88%). However in survey of TRAORE et al. (2012) no statistical differences in GP were noted between pork with DL_{48} below 2.8% and above 4%. BERTOL et al. (2017) suggested, that 87.33% of correctly classified carcasses with DL_{48} below and above 6% is possible to evaluate on the basis of linear discriminant function using HCW, backfat thickness and loin depth in conjunction with pH and temperature measured at 45 min after slaughter. Also, as reported by cited authors using adjusted regression equations on the basis of the same parameters 85.44% of correctly classified carcasses to DL_{48} threshold value of 6% and 80.32% to DL_{48} threshold value of 5% could be obtain. In study of KUSEC et al. (2007) pork loin samples with DL₄₈ threshold value of 5% was correctly classified in 62.18% and 77.31% on the basis of discriminant analysis and cluster analysis respectively.

CONCLUSION

Share of pork quality classes evaluated on the basis of different pH time-points and threshold values varied in population of (Landrace × Yorkshire) × Duroc fatteners. Frequent occurring of RFN did not correspond with lowered drip loss (Low DL within this meat samples classified as RFN did not exceed 50%). Therefore, pH-dependent methods of pork quality evaluation should be at least revised for their appropriate use in modern pork industry. Higher share of correctly classified meat samples with $DL_{48} \leq 4\%$ was noted if muscle metabolites threshold values measured 45 min. after slaughter were used. The most promising cut-off points were 45 µmol per g of muscle tissue both for glycogen and lactate level that allowed to correctly classify 83.82% of Low DL and 50% of High DL. Thereby use of glycogen and lactate threshold values in pre-rigor state could possibly be profitable for meat plants due to increase in accuracy of properly sorted carcasses with lowered drip loss. The potential of muscle metabolites use in pork quality evaluation is then high.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study nor in data collection, analyses and interpretation.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

BARBUT, S. et al. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. **Meat Science**, v.79, n.1, p.46–63, 2008. Available from: https://doi.org/10.1016/j. meatsci.2007.07.031>. Accessed: May, 14, 2020. doi: 10.1016/j. meatsci.2007.07.031.

BERGMEYER, H. U. Methods of Enzymatic Analysis. Academic Press: New York. 1978.

BERTOL, M. T. et al. Swine carcasses classified by degree of exudation and marbling content. **Pesquisa Agropecuaria Brasileira**, v.52, n.2, p.121–128, 2017. Available from: https://doi.org/10.1590/s0100-204x201700020006>. Accessed: May, 17, 2020. doi: 10.1590/s0100-204x2017000200006.

BOLER, D. D. et al. Ultimate pH explains variation in pork quality traits. **Journal of Muscle Foods**, v.21, n.1, p.119–130, 2010. Available from: https://doi.org/10.1111/j.1745-4573.2009.00171. x>. Accessed: Jun. 5, 2020. doi: 10.1111/j.1745-4573.2009.00171.x.

CAZADEY, H. P. et al. Comparison of different criteria used to categorize technological quality of pork. **Ciência Rural**, v.46, n.12, p.2241–2248, 2016. Available from: http://dx.doi.org/10.1590/0103-8478cr20160013>. Accessed: Jun. 14, 2020. doi: 10.1590/0103-8478cr20160013.

CHRISTENSEN, L. B. Drip loss sampling in porcine m. longissimus dorsi. **Meat Science**, v.63, n.4, p.469–477, 2003. Available from: https://doi.org/10.1016/S0309-1740(02)00106-7. Accessed: Feb. 22, 2020. doi: 10.1016/S0309-1740(02)00106-7.

CORREA, J. A. et al. 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**, v.18, n.1, p.67–77, 2007. Available from: https://doi.org/1 0.1111/j.1745-4573.2007.00066.x>. Accessed: Mar. 25, 2020. doi: 10.1111/j.1745-4573.2007.00066.x.

DALRYMPLE, R. H.; HAMM, R. A method for the extraction of glycogen and metabolites from a single muscle sample. **Journal of Food Technology**, v.8, n.4, p.439–444, 1973. Available from: https://doi.org/10.1111/j.1365-2621.1973.tb01730.x. Accessed: Feb. 25, 2019. doi: 10.1111/j.1365-2621.1973.tb01730.x.

DANIEL, C. R. et al. Trends in meat consumption in the United States. **Public Health Nutrition**, v.14, n.4, p.575–583, 2011. Available from: https://doi.org/10.1017/S1368980010002077. Accessed: Sep. 7, 2020. doi: 10.1017/S1368980010002077.

FAUCITANO, L. et al. Shelf life of pork from five different quality classes. **Meat Science**, v.84, n.3, p.466–469, 2010. Available from: https://doi.org/10.1016/j.meatsci.2009.09.017>. Accessed: Dec. 5, 2019. doi: 10.1016/j.meatsci.2009.09.017.

FILHO, R. D. A. T et al. Drip Loss Assessment by Different Analytical Methods and Their Relationships with Pork Quality Classification. Journal of Food Quality, v.6, p.1–8, 2017. Available from: https://doi.org/10.1155/2017/9170768. Accessed: Jul. 4, 2020. doi: 10.1155/2017/9170768.

FISCHER, K. Drip loss in pork: Influencing factors and relation to further meat quality traits. **Journal of Animal Breeding and Genetics**, v.124, n.s1, p.12–18, 2007. Available from: https://doi.org/10.1111/j.1439-0388.2007.00682.x. Accessed: Jul. 4, 2020. doi: 10.1111/j.1439-0388.2007.00682.x.

FUJII, J. et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. **Science**, v.253, n.5018, p.448–450, 1991. Available from: https://science.sciencemag.org/content/253/5018/448. Accessed: Mar. 13, 2020. doi: 10.1126/science.1862346.

GRAU, R.; HAMM, R. Eine einfache Methode zur Bestimmung der Wasserbindung in Muskel. **Naturwissenschaften**, v.4, p.29-30, 1953. Available from: https://link.springer.com/article/10.1007/ BF00595734>.

HUFF-LONERGAN, E.; LONERGAN, S.M. New frontiers in understanding drip loss in pork: recent insights on the role of postmortem muscle biochemistry. **Journal of Animal Breeding and Genetics**, v.124, n.1, p.19–26, 2007. Available from: https:// doi.org/10.1111/j.1439-0388.2007.00683.x. Accessed: Jan. 22, 2019. doi: 10.1111/j.1439-0388.2007.00683.x.

JOO, S. T. et al. Control of fresh meat quality through manipulation of muscle fiber characteristics. **Meat Science**, v.95, n.4, p.828–836, 2013. Available from: https://doi.org/10.1016/j. meatsci.2013.04.044>. Accessed: Apr. 14, 2020. doi: 10.1016/j. meatsci.2013.04.044.

KAPPER, C. et al. Moisture absorption early postmortem predicts ultimate drip loss in fresh pork. **Meat Science**, v.96, n.2A, p. 971–976, 2014. Available from: https://doi.org/10.1016/j. meatsci.2013.10.009>. Accessed: May, 5, 2020. doi: 10.1016/j. meatsci.2013.10.009.

KAUFFMAN, R. G. The effectiveness of examining early postmortem musculature to predict ultimate pork quality. **Meat Science** v.34, n.3, p.283–300, 1993. Available from: https://doi.org/10.1016/0309-1740(93)90078-V. Accessed: Nov. 15, 2019. doi: 10.1016/0309-1740(93)90078-V.

KAWASAKI, E. S. Sample preparation from blood cells and other fluids. In: INNIS, M.A; GELFAMD, D.H.; SNINSKY, J.J.; WHITE, T.J. **PCR protocols: A guide to methods and applications**. San Diego: Academic Press, 1990, p.146–152.

KIM, T. W. et al. Pork Quality Traits According to Postmortem pH and Temperature in Berkshire. **Korean Journal for Food Science of Animal Resources**, v.36, n. 1, p. 29–36, 2016. Available from: http://koreascience.or.kr/article/JAKO201607761295768.page. Accessed: Nov. 15, 2019. doi: 10.5851/kosfa.2016.36.1.29.

KNOX, B. L. et al. Relationships between Ultimate pH and Microbial, Chemical, and Physical Characteristics of Vacuum-

Packaged Pork Loins. Journal of Food Science, v. 73, n. 3, m104–m110, 2008. Available from: https://doi.org/10.1111/j.1750-3841.2008.00667.x. Accessed: Jun. 3, 2020. doi: 10.1111/j.1750-3841.2008.00667.x.

KOĆWIN-PODSIADŁA, M. et al. Pork quality and methods of its evaluation – a review. **Polish Journal of Food and Nutrition Sciences**, v.56, n.3, p.241–248, 2006. Available from: http://journal.pan.olsztyn.pl/PORK-QUALITY-AND-METHODS-OF-ITS-EVALUATION-A REVIEW,97943,0,2.html. Accessed: Jun. 27, 2020.

KOĆWIN-PODSIADŁA, M. et al. Glycogen content and the rate of glycolytic changes as the indicator of pork meat quality. In: 55th International Congress of Meat Science and Technology, 2009, Copenhagen, Prague. PE1.46. 2009. Available from: http://icomst-proceedings.helsinki.fi/papers/2009_01_54.pdf>. Accessed: Mar. 11, 2020.

KUSEC, G. et al. Discrimination between pork longissimus muscles with excessive and acceptable drip loss. In: 53rd International Congress of Meat Science and Technology, 2007, Beijing, China, v.4, p.67–68. Available from: http://icomstpoceedings.helsinki.fi/papers/2007_04_67.pdf>. Accessed: Mar. 11, 2020.

MATARNEH, S. K. et al. Net lactate accumulation and low buffering capacity explain low ultimate pH in the *longissimus lumborum* of AMPKγ3R200Q mutant pigs. **Meat Science**, v.110, p.189–195, 2015. Available from: https://doi.org/10.1016/j.meatsci.2015.07.023. Accessed: Mar. 6, 2020. doi: 10.1016/j.meatsci.2015.07.023.

MONIN, G.; SELLIER, P. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. **Meat Science**, v.13, n.1, p.49–63, 1985. Available from: https://doi.org/10.1016/S0309-1740(85)80004-8>. Accessed: Feb. 14, 2020. doi: 10.1016/S0309-1740(85)80004-8.

NAVEAU, J. et al. Proposition d'une méthode de mesuredu rendement technologique: la méthode Napole. **Techni-Porc**, v.8, p.7–13, 1985.

OTTO, G. et al. Different approaches of estimating economical values for drip loss as log normally distributed trait. **Livestock Science**, v.112, n.1-2, p.43–51, 2007. Available from: https://doi.org/10.1016/j.livsci.2007.01.155. Accessed: May, 19, 2020. doi: 10.1016/j.livsci.2007.01.155.

PIC 2003. **Pork Meat Quality**: Understanding Industry Measurements and Guidelines. [2016-11-25]. Available from: https://thepigsite.com/articles/pork-meat-quality-understanding-industry-measurements-and-guidelines>. Accessed: Feb. 7, 2020. doi: 10.1016/j.livsci.2007.01.155.

POHJA, N. S.; NINIVAARA, F.P. Die Bestimmung der Wasserbindung des Fleisches mittels der Konsandrückmethods. Fleischwirtschaft, v.9, p.193–195, 1957.

POSO, A. R.; PUOLANNE E. Carbohydrate metabolism in meat animals. **Meat Science**, v.70, n3, p.423–434, 2005. Available from: https://doi.org/10.1016/j.meatsci.2004.12.017. Accessed: May, 22, 2020. doi: 10.1016/j.meatsci.2004.12.017. POSPIECH, E. et al. Meat defects and their characteristic. In: **Pisula, A.; Pospiech, E. Meat - Science and technology base**. Warsaw: SGGW Publisher, Warsaw, 2011, pp. 233–243.

PRANGE, H. et al. Untersuchung zur Muskelfleisch-qualität beim Schwein. 3. Mitt.: Bedeutung der Fleischtemperatur unter besonderer Berücksichtigung der Transportform. Archiv für Experimentelle Veterinär Medizin Leipzig, v.31, n.2, p.337, 1977.

PRZYBYLSKI, W. et al. Jakość technologiczna i sensoryczna wadliwego mięsa wieprzowego. ŻYWNOŚĆ. Nauka. Technologia. Jakość, v.1, n.80, p.116–127, 2012. Available from: <http://bazekon.icm.edu.pl/bazekon/element/bwmeta1.element. ekon-element-000171309121>. Accessed: Apr. 21, 2020.

RAUW, W.M. et al. Meat production using four terminal pig lines. Journal of the Science of Food and Agriculture, v.83, n.14, p.1504–1510, 2003. Available from: https://doi.org/10.1002/jsfa.1568. Accessed: May. 22, 2020. doi: 10.1002/jsfa.1568.

RYBARCZYK, A. et al. The effect of DanAvl Duroc and Pulawska boars in crossbred with DanAvl hybrid on meat quality of finishing pigs. **Agricultural and Food Science**, v.27, n.3, p.159–167, 2018. Available from: https://doi.org/10.23986/afsci.70934>. Accessed: May, 26, 2020. doi: 10.23986/afsci.70934.

SCHAFER, A. et al. Physiological and structural events post mortem of importance for drip loss in pork. **Meat Science**, v.61, n.4, p.355–366, 2002. Available from: https://doi.org/10.1016/S0309-1740(01)00205-4>. Accessed: Apr. 5, 2020. doi: 10.1016/S0309-1740(01)00205-4.

SCHEFFLER, T. L. High glycolytic potential does not predict low ultimate pH in pork. **Meat Science**, v.95, n.1, p. 85–91, 2013. Available from: https://doi.org/10.1016/j.meatsci.2013.04.013. Accessed: Apr. 5, 2020. doi: 10.1016/j.meatsci.2013.04.013.

TARCZYŃSKI, K. et al. pH measured 24 hours post mortem should not be regarded as ultimate pH in pork meat quality evaluation. **South African Journal of Animal Science**, v.48, n.6, p.1009–1016, 2018. Available from: https://www.ajol.info/index.php/sajas/article/view/183081>. Accessed: Sep. 3, 2020. doi: 10.4314/sajas.v48i6.2.

TRAORE, S. et al. Higher drip loss is associated with protein oxidation. **Meat Science**, v.90, n.4, p.917–924, 2012. Available from: https://doi.org/10.1016/j.meatsci.2011.11.033. Accessed: Jun. 6, 2020. doi: 10.1016/j.meatsci.2011.11.033.

TROY, D. J.; KERRY, J.P. Consumer perception and the role of science in the meat industry. **Meat Science**, v.86, n.1, p.214–226, 2010. Available from: https://doi.org/10.1016/j.meatsci.2010.05.009. Accessed: Jun. 6, 2020. doi: 10.1016/j.meatsci.2010.05.009.

ZENG, Z. et al. Effect of dietary supplementation with mulberry (Morus alba L.) leaves on the growth performance, meat quality and antioxidative capacity of finishing pigs. **Journal of Integrative Agriculture**, v.18, n.1, p.143–151, 2019. Available from: https://doi.org/10.1016/S2095-3119(18)62072-6. Accessed: Jun. 15, 2020. doi: 10.1016/S2095-3119(18)62072-6.

ZYBERT, A. et al. Relationship of glycogen and lactate concentrations as a pork quality indicator. **South African Journal of Animal Science**, v.50, n.1, p.26–37, 2020. Available from: https://www.ajol.info/index.php/sajas/article/view/194884>. Accessed: Jun. 15, 2020. doi: 10.4314/sajas.v50i1.4.

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