RELATION BETWEEN THE POST MORTAL PH AND ELECTRICAL CONDUCTIVITY (EC) DEVELOPMENT, THE DRIP LOSS AND THE MITOCHONDRIAL RESPIRATORY ACTIVITY IN THE PORCINE LONGISSIMUS MUSCLE OF DIFFERENT PIG GENETICS

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Introduction

The ability of fresh meat to retain moisture is one of the most important quality characteristics of raw products. It has been estimated that much of the pork produced unacceptably high purge or drip loss (Huff-Lonergan and Lonergan, 2005). The situation in muscle cells upon slaughter is related to the focal anoxia and ischemia resulting, for example, in the activation of calcium-dependent processes (e.g. contraction, glycolysis), acidification, partial denaturation and shrinkage of the myofibrillar proteins and reduction in the amount of water that can be attracted and held by the muscular, mainly myofibrillar proteins (Bertram et al., 2004; Huff-Lonergan and Lonergan, 2005). The most severe drip loss could be found in pale, soft and exudative (PSE) meat from pigs that have a mutation in the ryanodine receptor (RyR)/ calcium-release channel (CRC) of the sarcoplasmic reticulum (SR) (Fuji et al., 1991). In the muscle cells of these pigs the control of the calcium release is impaired and the release of this cation from the SR to the sarcoplasm accelerated - especially after physical stress before slaughter. Due to the relation between calcium and the muscle-to-meat-transition process higher concentrations of this ion cause more rapid contractions, an increase in the rate of muscle metabolism and an accelerated acidification of the tissue. This rapid pH decline leads to a reduced water-holding capacity (WHC) of the tissue (Huff-Lonergan and Lonergan, 2005). The alteration is comparable with the malignant hyperthermia syndrome (MHS) in human. For this reason pigs with the described mutation are also called "MHS positive". Usually the calcium concentration in the sarcoplasm is reduced by ATP-dependent transporter proteins in the SR (SERCA). The mitochondria also influence the sarcoplasmic calcium concentration via calcium-dependent in- and efflux-systems in the mitochondrial membrane (Gunther et al., 2004). Considering this in the present work the changes in the mitochondrial function – especially of the respiration capacity – was investigated in pigs in relation to the post mortal pH and electrical conductivity (EC) development and the drip loss. The objective was to obtain a further understanding of how the muscle mitochondria influence the muscle-to-meat-transition.

Animals, Materials and Methods

The three pure-bred genetics Pietrain (homozygote MHS negative (PiNN), N=10), Pietrain (homozygote MHS positive (PiPP), N=10) and Duroc (homozygote MHS negative (Du, N=10) as well as a F2-cross-bred population of Du and PiNN (DuPi, N=10) were used in the study. Both females and castrated males were used. 24 hours before slaughter a biopsy was collected from the M. longissimus thoracis (LM) at the 13./14. Thoracic vertebra (Th) and prepared for the analysis of the mitochondrial respiration activity. The pigs were slaughtered in an experimental abattoir. After electrical stunning (250 V, 1.3 A, 6 s) and exsanguination the carcasses were scalded at 62°C for 3 min, cleaned and eviscerated within 30 min. Within 60 min the carcasses were transferred to a chilling room and stored at 4°C. Immediately after stunning and before exsanguination a biopsy was collected again from the LM at the 13./14. Th and prepared for the analysis of the mitochondrial respiration activity. The pH (Portamess, Knick, Germany) and electrical conductivity (EC) (LF Star, Matthäus, Germany) were determined at the section of the LM 45 min (13./14. Th), 12 h (12./13. Th) and 24 h (14./15. Th) after stunning. 12 h after slaughter a third muscle sample was removed from the LM at the 13./14. Th for the final analysis of the mitochondrial respiration. 24 h after slaughter a slice of the LM (2.5 cm) was excised at the 10./11. Th. After trimming of the adjacent tissue the muscle samples were weighed and placed in a container equipped with a lid. After storage for 48 h at 4°C the sample was reweighed and the drip loss calculated as the weight loss percentage (Bertram et al., 2004). The mitochondrial respiration activity was determined with an Oxygraph (Oroboros, Austria) equipped with a Clark-electrode according to Kuznetsov et al. (2003) using the skinned fiber technique. Mitochondrial respiratory activity (state-3 and state 4 respiration) was analysed using the substrate pyruvate/ malate. The data were analysed with the software Statistica 7.1 (StatSoft, Germany) using the GLM procedure. The statistical model included the effects genetic, gender and slaughter date. A significant effect of the gender and slaughter date could not be calculated. Significance was analysed with the Fisher LSD test considering $P<0.05$.

Results and Discussion

Analysis of the pH 45 min after slaughter showed that the genetic PiPP had significantly (P<0.05) lower pH values in comparison to the other genetics. During ageing of the meat the pH values declined to comparable results of 5.7 in all investigated genetics (Tab. 1). An exception was the significant (P<0.05) difference between the PiPP and PiNN genetic 12 h after slaughter which seems to be a random result. With regard to the EC and drip loss values the PiPP genetic showed at all points of time significantly (P<0.05) higher results in comparison to the other genetics that did not differ significantly (P<0.05) (Tab. 1). The presented data are in accordance with other results showing that the mutation in the RyR of the SR has a negative effect on the muscle-to meat transition especially on the pH decline and the drip loss (Huff-Lonergan and Lonergan 2005). The correlation analysis between the drip loss and the pH or EC values showed significant correlations between the drip loss and all determined pH and EC values (data not shown). The r-values of the pH decreased from r = -0.66 (45 min) to r = -0.44 (24 h) whereas the correlation between the EC and the parameter drip loss remained nearly constant during ageing (45 min: r = 0.76; 12h: r = 0.75; 24h: r = 0.78). However, the high correlation between the drip loss and the early post mortal EC (EC 45 min) as well as the late pH values (pH 24 h) has to be critically discussed as is in contrast to other investigations. A reason might be the low amount of investigated animals. Further investigations are necessary to clarify this discrepancy.

Parameter	DuPi (N=10)	PiNN (N=10)	PiPP (N=10)	Du (N=10)
pH 45 min	6.3 ± 0.08^{a}	6.3 ± 0.08^{a}	5.6 ± 0.08^{b}	6.2 ± 0.08^{a}
pH 12 h	5.7 ± 0.05^{ab}	5.8 ± 0.05^{b}	5.6 ± 0.05^{a}	5.7 ± 0.05^{ab}
pH 24 h	5.7 ± 0.05^{a}	5.7 ± 0.05^{a}	5.7 ± 0.05^{a}	5.7 ± 0.05^{a}
EC 45 min [mS/cm]	4.4 ± 0.8^{a}	5.8 ± 0.84^{a}	17.5 ± 0.8^{b}	5.8 ± 0.8^{a}
EC 12 h [mS/cm]	6.1 ± 0.63^{a}	5.5 ± 0.66^{a}	14.9 ± 0.63^{b}	6.6 ± 0.63^{a}
EC 24 h [mS/cm]	7.3 ± 0.58^{a}	6.5 ± 0.60^{a}	13.4 ± 0.58^{b}	7.0 ± 0.58^{a}
Drip Loss [%]	3.0 ± 0.49^{a}	2.6 ± 0.51^{a}	5.9 ± 0.49^{b}	2.5 ± 0.49^{a}

Tab. 1: Least square means (LSM) and standard errors of the pH and EC values determined in the M. longissimus thoracis (LM) of different pig genetics 45 min, 12 h and 24 h after slaughter. The drip loss was analyzed with slices of the LM between 24 h and 72 h after slaughter.
[a,b] LSM with different superscripts differ significantly (P<0.05).

With regard to the mitochondrial respiratory activity in the LM samples of the different pig genetics it could be shown that the state-3 respiration using the substrates malate/ pyruvate as well as the state-4 respiration was comparable between the investigated genetics not only 24 h before slaughter, but also immediately after stunning (Tab.2). However, 12 h after slaughter the muscle fibers of the genetic PiPP had significantly (P<0.05) reduced pyruvate/malate dependent state-3 respirations whereas the other genetics had comparable respiratory activities. The state-4 respiratory activity was comparable between the pig genetics (Tab.2). From the results it could be suggested that the investigated pig genetics had a comparable oxidative metabolism before and during slaughter but during muscle-to-meat-transition a clear correlation between the development of PSE meat and the mitochondrial function could be shown. As it is not clear if the reduced mitochondrial respiratory activity is related to the rapid pH decline or if the disturbed mitochondrial function negatively influences the post mortal metabolism especially the calcium-related processes further investigations are under progress to clarify the results.

Mitochondrial respiration in nmol O2/min*mg	DuPi (N=10)	PiNN (N=10)	PiPP (N=10)	Du (N=10)
State-3 respiration (- 24 h)	5.0 ± 0.8	5.0 ± 0.8	7.1 ± 0.8	6.9 ± 0.8
State-4 respiration (- 24 h)	1.5 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
State-3 respiration (0 min)	6.5 ± 0.5	5.2 ± 0.6	6.3 ± 0.6	6.1 ± 0.6
State-4 respiration (0 min)	1.3 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
State-3 respiration (12 h)	5.0 ± 0.6a	3.5 ± 0.6a	1.6 ± 0.6b	4.9 ± 0.6a
State-4 respiration (12 h)	1.3 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	1.4 ± 0.2

Tab. 2: Least square means (LSM) and standard errors of the pyruvate–dependent state-3 and state-4 mitochondrial respiration determined in skinned muscle fibers of the M. longissimus thoracis (LM) 24 h before slaughter (-24 h), immediately after stunning (0 min) and 12 h after slaughter (12 h) depending on the pig genetic.
[a,b] LSM with different superscripts differ significantly (P<0.05).

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