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Adenine nucleotide concentrations and glycolytic enzyme activities in longissimus muscle samples of different pig genotypes collected before and after slaughter

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ABSTRACT

Longissimus muscle samples from the pig genotypes Duroc (Du), Pietrain (MHS homozygote negative (PiNN), positive (PiPP)) and a Duroc-Pietrain crossbreed (DuPi) were analyzed. The PiPP samples showed a faster pH drop and higher electrical conductivity, drip loss and lightness values. Before slaughter the concentrations of the adenine nucleotides were comparable between the genotypes, but 40 min after slaughter (p.m.) the ATP concentrations decreased and IMP increased, to a higher extent in the PiPP pigs. The nucleotide values of the 12 h p.m. samples were again comparable. Activities of glycogen phosporylase (GP), phosphofructokinase (PFK) and lactate dehydrogenase (LDH) were nearly similar before slaughter. Forty minutes after slaughter the LDH activities increased in all pigs and the PFK activities in all genotypes but not in the PiPP. GP results were rather inconsistent indicating an earlier activation of this enzyme. The study showed that the reduced meat quality in the PiPP pigs is accompanied with rapid ATP degradation and accelerated enzyme activation.

1. Introduction

After slaughter of pigs ischemia and accumulation of sarcoplasmic calcium in the muscles result in contraction of myofibrils, transition to glycolysis and accumulation of lactate (Binke, 2004). Several publications showed that during this post mortem period ATP is splitted to ADP and subsequently to AMP and IMP (Battle, Aristoy, & Toldra, 2001; Copenhafer, Richert, Schinckel, Grant, & Gerrard, 2006; Fernandez, Neyraud, Astruc, & Sante, 2002; Hamm, 1977; Shen et al., 2006; Shen, Underwood, Means, McCormick, & Du, 2007). This breakdown of adenosine nucleotides occurs faster in meat with low quality e.g. in animals with a mutation in the gene coding for the ryanodine receptor (RyR) of the sarcoplasmic reticulum (Battle et al., 2001; Battle, Aristoy, & Toldra, 2000; Copenhafer et al., 2006; Shen et al., 2007) but also in RyR negative pigs after transport stress (Shen et al., 2006). This low quality meat showed higher lightness and drip loss values and an accelerated pH decline (Shen et al., 2006, 2007). In the latter investigations the authors also showed that the activities of glycolytic enzymes like glycogen phosphorylase or, indirectly, phosphofructokinase – analyzed during post mortem period – are related to the meat quality and adenine nucleotide breakdown. With regard to the publications by Shen et al. (2006, 2007) the objective of this study was to evaluate the adenine nucleotide breakdown in relation to different glycolytic enzyme activities in different pig genotypes thereby considering also animals with the RyR mutation. The novelty of this study was not only the analysis of the nucleotide concentrations and glycolytic enzyme activities in the same samples, but also the examination of results collected before slaughter by biopsy and after slaughter.

2. Material and methods

2.1. Animals, husbandry, sample collections and analysis of meat quality traits

The experiments were conducted following the German and European animal welfare regulations for animal husbandry, transport and slaughter. For the collection of muscle biopsies as an approvable animal experiment an official permission from the responsible authorities for animal welfare was received (33.42502/01-47.05).

The pig genotypes Duroc (Du), Pietrain (MHS homozygous negative (PiNN)), Pietrain (MHS homozygous positive (PiPP)) and the F2-Duroc-Pietrain-Resource Population of the University of Bonn (MHS homozygote negative, DuPi) (N = 32 per genotype) were fattened and transported as described previously (Werner, Natter, Schellander, & Wicke, 2010a). The mean carcass weight of the genotypes was with a value of 83.2 kg comparable. The lean meat percentage values of the PiPP (67.3%) were significantly higher followed by the results of the PiNN (63.9%) and Du (62.0%) and the DuPi pigs (58.4%). Twenty-four hours before slaughter (ante mortem (a.m.)) a shot biopsy was removed from the longissimus muscle (LM) between the 13/14th thoracic vertebra (Th) (Werner et al., 2010a). The collected muscle samples ($T_{-24\ h}$) were frozen in liquid nitrogen and stored at −80 °C until analysis. On the following day the pigs

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were slaughtered in the experimental abattoir of the Department of Animal Sciences in Goettingen (Werner et al., 2010a). The pH (pH Star, Matthäus GmbH, Poettmes, Germany) and electrical conductivity (EC) (LF Star, Matthäus) of the LM was determined 45 min and 12 h after slaughter (p.m.). Muscle samples were collected 40 min ($T_{40\ min}$) and 12 h p.m. ($T_{12\ h}$) and stored until nucleotide and enzyme analysis as described above. After 24 h a part of the LM was removed from the carcass and the color (L*a*b*) values and drip loss values determined according to Werner et al. (2010a).

2.2. Nucleotide and enzyme activity analysis

The concentrations of the nucleotides ATP, ADP, AMP and IMP were determined according to Morzel and van de Vis (2003) with modifications. The modifications were that the homogenized meat was centrifuged for 5 min at 20.000 *g*, the supernatant neutralized with potassium hydroxide and again centrifuged. The supernatant was stored at −80 °C until nucleotide analysis. The HPLC system consisted of a Merck-Hitachi L7100 pump, L7200 autosampler with a variable loop (injection volume: 20 µl), L4250 UV detector (λ = 254 nm) and a LiChroCart Licrosphere RP8 (250 × 4.6 mm, 5 µl) maintained at 25 °C with a L7360 column oven. The degassed isocratic mobile phase (0–3 min: 1.0 ml/min; 4–25 min: 1.5 ml/min) consisted of 100 mM KH_2PO_4, 1.44 mM tetrabutylammonium hydrogen sulfate and 0.5% methanol adjusted to pH 7.0.

The enzyme activities of glycogen phoshorylase (GP), phosphofructokinase (PFK) and lactate dehydrogenase (LDH) were determined according to Werner, Natter, and Wicke (2010b).

2.3. Chemicals

All chemicals were purchased from Sigma-Aldrich GmbH, Taufkirchen, Germany, unless otherwise indicated.

2.4. Statistical analysis

The data were analyzed with the software Statistica 7.1 (StatSoft, Hamburg, Germany) using the GLM procedure. The statistical model included the effects of genotype, gender, carcass weight and slaughter date. A significant effect of the gender, carcass weight and slaughter date was not found. Significance was analyzed with the TUKEY test considering $P<0.05$.

Differences between the nucleotide concentrations and the enzyme activities at the three different collections times (−24 h, 40 min, 12 h) were analyzed with the *t*-test of dependent measures considering $P<0.05$.

3. Results

3.1. Meat quality traits

The meat quality characteristics depending on the pig genotype are presented in Table 1. The pH-values determined 45 min and 12 h p.m. were lower ($P<0.05$) in the PiPP animals in comparison to the other genotypes. The Du, PiNN and DuPi pigs had similar pH values except of the significantly lower $pH_{12\ h\ p.m.}$ of the DuPi pigs.

The EC values of the PiPP animals were higher ($P<0.05$) at both determination times (45 min, 12 h p.m.) in comparison to the other pig genotypes.

The color (L*a*b*) values were comparable between the pig genotypes DuPi, PiNN and Du, differing clearly ($P<0.05$) from the PiPP pigs that had higher lightness (L*, 51.9), redness (a*, 9.12) and yellowness (b*, 0.33) ($P<0.05$).

The highest drip loss values could be determined in meat from the PiPP animals differing ($P<0.05$) from the results of all other strains.

Table 1
Least square means (LSM) and standard deviations (SD) of different meat quality traits determined in the longissimus muscle depending on the pig genotype.

	DuPi (n = 32)		PiNN (n = 32)		PiPP (n = 32)		Du (n = 32)	
	LSM	SD	LSM	SD	LSM	SD	LSM	SD
pH 45 min	6.20^{a}	0.22	6.24^{a}	0.24	5.59^{b}	0.26	6.19^{a}	0.32
pH 12 h	5.69^{b}	0.17	5.82^{a}	0.18	5.59^{c}	0.11	5.79^{a}	0.16
EC[1] 45 min	5.20^{b}	1.19	5.53^{b}	1.72	14.36^{a}	5.99	5.58^{b}	1.47
EC[1] 12 h	6.46^{b}	1.73	5.64^{b}	1.86	13.11^{a}	2.79	6.72^{b}	1.89
L* 24 h p.m.	47.89^{b}	2.36	47.87^{b}	2.43	51.91^{a}	3.27	47.97^{b}	2.72
a* 24 h p.m.	8.06^{b}	1.01	7.94^{b}	1.05	9.12^{a}	1.11	8.28^{b}	1.44
b* 24 h p.m.	-0.66^{b}	1.26	-1.01^{b}	1.44	0.33^{a}	1.7	-1.13^{b}	1.48
Drip loss [%][2]	2.67^{b}	1.34	2.79^{b}	1.22	6.09^{a}	1.96	2.69^{b}	1.16

[abc] Within a row, means without a common superscript letter differ ($P<0.05$).
[1] EC = electrical conductivity in mS/cm.
[2] Drip loss determined between 24 h and 72 h after slaughter.

The genotypes DuPi, PiNN and Du had similar drip loss values ($P>0.05$).

3.2. Nucleotide and enzyme activity analysis

The results of the adenine nucleotide analysis are presented in Table 2. In the meat samples collected 24 h before slaughter the nucleotide concentrations were nearly comparable. Exceptions were the significantly higher ADP values in the PiPP pigs in comparison to the DuPi animals and the generally lower AMP concentrations ($P<0.05$) in the samples collected from the genotypes PiNN and PiPP.

After slaughter of the animals the meat ATP concentrations generally decreased ($P<0.05$) but to the highest extent in the PiPP pigs resulting in very low ATP concentrations that differ from the results of all other genotypes. The animals from the PiNN genotype had the highest ATP values 40 min p.m. differing from the results of the PIPP and Du. The reduction of the ATP content in all pigs was accompanied with a significant increase of the ADP concentrations in the DuPi and Du pigs. Despite this increase, the ADP values of all genotypes were comparable in 40 min p.m. samples. The AMP

Table 2
Least square means (LSM) and standard deviations (SD) of the adenine nucleotide concentrations analyzed in longissimus muscle samples collected 24 h before slaughter (ante mortem (a.m.)) and 40 min and 12 h post mortem (p.m.) depending on the pig genotype.

	DuPi (n = 32)		PiNN (n = 32)		PiPP (n = 32)		Du (n = 32)	
	LSM	SD	LSM	SD	LSM	SD	LSM	SD
ATP concentrations [µmol/g muscle]								
24 h a.m.	7.56^{ax}	0.77	7.18^{ax}	1.1	7.61^{ax}	1.05	7.13^{ax}	0.98
40 min p.m.	3.97^{aby}	1.56	4.81^{ay}	0.9	0.93^{cy}	1.18	3.36^{by}	1.71
12 h p.m.	0.04^{abz}	0.06	0.13^{az}	0.20	0.01^{bz}	0.01	0.08^{abz}	0.128
ADP concentrations [µmol/g muscle]								
24 h a.m.	0.61^{by}	0.09	0.67^{abx}	0.14	0.72^{ax}	0.09	0.65^{aby}	0.09
40 min p.m.	0.88^{ax}	0.64	0.69^{ax}	0.16	0.79^{ax}	0.44	0.78^{ax}	0.19
12 h p.m.	0.52^{bz}	0.11	0.61^{ax}	0.16	0.57^{aby}	0.09	0.56^{abz}	0.18
AMP concentrations [µmol/g muscle]								
24 h a.m.	1.03^{ax}	0.25	0.77^{bx}	0.23	0.72^{bx}	0.24	1.01^{ax}	0.3
40 min p.m.	1.09^{ax}	0.25	0.81^{bx}	0.22	0.72^{bx}	0.21	1.08^{ax}	0.26
12 h p.m.	0.82^{ay}	0.26	0.79^{ax}	0.21	0.23^{by}	0.16	0.92^{ay}	0.32
IMP concentrations [µmol/g muscle]								
24 h a.m.	0.13^{ax}	0.15	0.29^{ax}	0.92	0.12^{ax}	0.06	0.16^{ax}	0.12
40 min p.m.	2.02^{bcy}	1.59	1.10^{cy}	1.01	6.04^{ay}	1.96	3.05^{by}	2.08
12 h p.m.	6.75^{az}	0.56	6.77^{az}	0.56	6.77^{az}	0.92	6.64^{az}	0.90

[abc] Within a row, means without a common superscript letter differ ($P<0.05$); [xy]Within a column, means without a common superscript letter within the same adenine nucleotide differ ($P<0.05$).