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Ante-mortem 31P NMR study of skeletal muscle metabolism on entire and on a muscle biopsy in Heterozygotes and MH Normal pigs

R. Lahucky[1], G. Kohn[2], U. Baulain[2], M. Henning[2], E. Kallweit[2], P. Demo[1], D. Vasicek[1], N. Pronayova[3]

[1]Research Institute of Animal Production, Nitra, Slovak Republic
[2]Institut fur Tierzucht und Tierhalten (FAL), Mariensee, Germany
[3]Chemical College, Slovak Technological University, Bratislava, Slovak Republic

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Background:
It is now possible to detect heterozygotes using the polymerase chain reaction, followed by restriction endonuclease, and electrophoretic detection of modified DNA fragments (Fuji et al., 1991, Houde and Pommier, 1993). In pig production, crossing between normal sows and m boars is expected to give a large yield and lower fat (Zhang et al., 1992). In practice, however, both heterozygotes and normal pigs may develo PSE meat (Jensen and Barton-Gade, 1985, Pommier and Houde, 1993) with poor water holding capacity (WHC), (Cheah et al., 1994). The advantages of the DNA-based test are that it is highly accurate and able to identify heterozygotes but is not sufficient to elicit all the MH susceptibility and to explain all PSE meat quality occurence (Cheah et al., 1994). It looks to be important modulating factors of the MH syndrome that must be present for full expression of the syndrome or if it is absent it is not observed (Fletcher et al., 1993). It was shown that capacity of the muscle energetic metabolism (Chance et al., 1986) could be very important factor influencing and modulating variation in the expression of the MH syndrome and occurence of PSE meat quality. Phosphorus NMR spectroscopy has been applied in studies on skeletal muscle energetic metabolism in vivo (Moesgaard et al., 1995, Scholz et al., 1995) also in vitro on bioptates (Miri et al., 1989, Shen et al., 19 Lahucky et al., 1993).

Objectives:
The aim of present study (performed in two experiments) was to determine energetic metabolism on entire pigs (**experiment 1**) and on a mu biopsy (**experiment 2**) in heterozygotes and normal pigs using 31P NMR spectroscopy measurements.

Material and methods:
DL pigs (18.5 - 55 kg live weight) were used in experiment 1. Twenty pigs (11 Duroc x Pietrain, 7 Duroc, 2 Slovak Meat White) were used in experiment 2. MH genotypes (normal, monomutant) were identified by DNA gene test (a ryanodine receptor - RYR 1 gene test using polymera chain reaction technique - PCR), (Fujii et al., 1991).
To prevent movement during NMR measurements (experiment 1) pigs were anesthetized by intra muscular administration of Tilest and after positioning the pigs oxygen was administered. In vivo after obtaining five reference spectra pigs were exposed via face mask to halothane at concentration of 2% in the semiopen system with oxygen (100 %) flow 3L/min. The maximum time of halothane exposure was limited to 20 minutes. But the halothane administration was stopped earlier before reaching the time limit after observing significant changes in the PCr pea in consecutive spectra. For carcasses (slaughter weight for normal pigs average 66.3 kg, heterozygotes 58.4 kg), post mortem changes (30 min 120 min) of inorganic phosphate (P_i), phosphocreatine (PCr), adenosine triphosphate (ATP) were observed during the experiment (Biospec 1.5/100, Bruker, Karlsruhe) and pH value were calculated (Moon, Richards, 1983).
At about 80 kg live weight (experiment 2), a biopsy sample of approx. 1 g was taken by efficient spring loaded biopsy (BIOTECH, Nitra) from the right side of Longissimus dorsi muscle (LD). The biopsy sample was introduced into a 10 mm diameter tube filled with deuterated water (D2O) for NMR measurements for 50-60 min. The 31P NMR spectra were recorded at 121 Mhz on a VXR 300 (Varian) spectrometer. The pro temperature was maintained at 39° C.The time of accumulation per spectrum was 7.6 min. The levels of the total content of phosphorus compounds were expressed in percentage of the total content of phosphorus compounds. After finishing of NMR measurements (55-60 min) f from bioptate was also directly determined by combined glass electrode. The pigs were killed by electrostunning and exsanguination at about 105 kg live weight. Samples were taken from LD at 1 hour after death for determination of pH (homogenisation of muscle in 5 mM iodoacetate pH 1 hour and 48 hour post-mortem were also determined directly on muscle tissue using combined glass electrode. The day after slaughter we cnductivity, external reflectance (520 and 630 nm) and drip loss determined on LD muscle.
The results are expressed as means ± s.e. and compared using t-tests.

Results and discussion:
Experiment 1: Plots of PCr/P_i versus time obtained from the spectra of different MH genotypes have shown significant differences before the halothane exposure (Tab 1). The energy states characterised by PCr/P_i and PCr/ATP were at heterozygotes higher to normal pigs.
In agreement to others (Decaniere et al., 1993) the higher basal intracellular ratio PCr/Pi or PCr concentrations observed in heterozygotes (monomutant) might reflect a different fibre composition and/or an „endogenous" effect as a consequence of a higher than normal sensitivity connected with an occurence of one allele (monomutant) of mutated of RYR 1 gene. At experimental condition (using 2.0 % vol. Hal. and 20 min administration) it was not able to find differences between heterozygotes and normal pigs during and after administration of halothane. As follows from post mortem results (Tab 2) using kinetic 31P NMR measurements were found significant differences between heterozygotes and normal pigs in rate of PCr breakdown and pH changes. Results confirmed that slaughtering could exagerate breakdown of PCr and glycolytic process in different level in heterozygotes and normal pigs but inspite this normal quality of meat is expected also from heterozygotes at light slaughter weight (below 70 kg l.w.).
Experiment 2: To express the rate of the phosphorylated compounds, we used the ratio of either PCr or the sum of PCr and ATP to the sum of inorganic phosphate (Pi) and sugar phosphate (SP). In order to be able to differentiate heterozygous pigs with propensity to produce normal and PSE meat, we decided to use mean value for pH (1h post mortem, in homogenate) to devide all pigs in experiment. Results from Table 3

showed the heterozygote pigs that produced meat with pH 1 h > 6.0 were closer to homozygous negative (normal) pigs. Significant differences ($P < 0.01$, $P < 0.05$) in all ante- and post-mortem traits were obtained between the PSE (pH < 6.0) heterozygous group and normal pigs (except reflectance and ultimate pH). There were also some significant differences between the PSE heterozygous group and normal heterozygous pigs in ante mortem and mainly in post mortem values. In our experiment significant difference ($P < 0.05$) between the PSE heterozygous group with pH < 6.0 and heterozygous group with pH > 6.0 were observed in the biopsy data of ratio PCr/(Pi + SP), (10 min). It seems the biopsy values for F (fluid volume) and pH (F) introduced earlier by Cheah et al. (1995) are better for selecting Nn pigs with superior WHC. The heterozygotes in some experiments could be closer to normal pigs as was also recently shown by Lahucky et al. (1997) and the possibility the incidence of PSE in Nn pigs could be influenced by poor pre- and/or post-slaughter management was discussed by Cheah et al., (1995).

Conclusions:

Slaughtering could exagerate breakdown of PCr and glycolytic process in different level in heterozygotes and normal pigs also at light (below 70 kg l.w.) slaughter weight (experiment 1).

Results from ante mortem NMR measurements (experiment 2) supported the possibility of a biopsy to predict of post mortem muscle metabolism rate and to differenciate PSE animals connected with the occurence of mutation on RYR 1 gene of pigs.

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Tab 1 Ratio PCr/Pi in rest biceps femoris

Genotyp	PCr/Pi	PCr/ATP
NN (n=12)	7.17 ± 1.64	3.67 ± 0.61
Nn (n=16)	8.56 ± 2.30	3.90 ±0.68

Differences between genotypes are significant at P <0.05

Tab 2 Kinetic parameters for post mortem PCr and Pi biceps femoris

Genotyp	$k(min^{-1})(x\ 10^2)$ PCr	Pi	$t_{1/2}$(PCr) min
NN (n=9)	2.46 ± 0.37	1.47 ± 0.60	28.1 ± 4.9
Nn (n=5)	3.17 ± 0.34	0.72 ± 0.43	21.9 ± 2.6

Table 3
Evaluation of PCr/(Pi + SP) and (PCr + ATP)/(Pi + SP) in biopsies and meat quality of heterozygous and normal pigs

	min	Nn pH < 6.0 (n = 7) x ± s		Nn pH > 6.0 (n = 6) x ± s		NN (n = 7) x ± s	
PCr/(Pi + SP)	10	0.65	0.3^a	0.99	$0.1^{b,c}$	1.26	0.3^c
	30	0.11	0.1^a	0.15	0.1^a	0.25	0.1^b
	50	0.05	0.0^a	0.07	$0.0^{a,b}$	0.13	0.0^b
(PCr + ATP)/ (Pi + SP)	10	1.40	0.2^a	1.71	$0.2^{a,b}$	2.00	0.4^b
	30	0.39	0.1^a	0.44	0.1^a	0.62	0.1^b
	50	0.18	0.1^a	0.23	$0.1^{a,b}$	0.33	0.1^b
Post mortem pH (iodoacet.)	1h	5.73	0.2^a	6.13	0.1^b	6.33	0.1^c
Conductivity	24h	11.30	2.2^a	5.50	1.8^b	4.14	0.9^b
Drip loss (%)	48h	7.76	0.4^a	6.61	3.7^b	5.30	0.9^c

Means with different superscripts are different at P < 0.05