ANTE-MORTEM MUSCLE METABOLISM ASSESSED BY 31P NMR SPECTROSCOPY AND MEAT QUALITY IN HETEROZYGOTES AND MH NORMAL PIGS.

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INTRODUCTION

Elimination of mutation in the gene controlling the calcium release chanel (Ryanodine receptor, RYR 1) of the sarcoplasmic reticulum is now essentially possible (Fujii et al., 1991). It is now also possible to detect heterozygotes using the polymerase chain reaction, followed by restriction endonuclease, and electrophoretic detection of modified DNA fragments (Houde and Pommier, 1993). 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 the 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 previously been applied in studies on skeletal muscle energetic metabolism also in vitro on bioptates (Miri et al., 1989, Shen et al., 1992, Lahucky et al., 1993).
The aim of present study was to investigate further the possibility of predicting the intensity of post-mortem muscle metabolism and ultimate meat quality in heterozygotes and normal pigs from 31P NMR measurements performed intra vitam on a muscle biopsy.

MATERIAL AND METHODS.

Twenty pigs (11 Duroc x Pietrain, 7 Duroc, 2 Slovak Meat White) were used in experiment. At about 80 kg live weight, a biopsy sample of approx. 1 g was taken by efficient spring loaded biopsy (Kovac et al., 1992) 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 probe temperature was maintained at 39° C. The total 31P spectrum was recorded with a sweep width of 3932.4 Hz and a 45 pulse of 35.0 mikrosec. The recycle time was 0.8 s. Each spectrum was a result of 512 transients. An exponentional line broadening of 20 Hz was used as internal reference at -2.47 ppm. 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) pH 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 were cnductivity, external reflectance (520 and 630 nm) and drip lossdetermined on LD muscle.
The results are expressed as means ± s.e. and compared using t-tests. The correlation coefficient (r) were also calculated.

RESULTS AND DISCUSSION.

To express the rate of change of the phosphorylated compounds, we used the ratio of either PCr or the sum of PCr and ATP - both decreasing with time - to the sum of inorganic phosphate (Pi) and sugar phosphate (SP) - both increasing with time. The rate of changes in phosphorus compounds from a biopsy of heterozygous and normal pigs are shown in Tables 1. Significant differences ($P < 0.01$, $P < 0.05$) of 31P NMR characteristics - ratios PCr/(Pi + SP) and (PCr + ATP)/(Pi + SP) of LD muscle were observed between heterozygotes and normal pigs. In every case PCr and ATP decreased with time, whereas (Pi + SP) increased as was earlier shown in bioptate of halothane positive and negative pigs (Miri et al., 1989, Lahucky et al., 1993).
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 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 (Table 2) 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), but not in pH bioptate. It seems the biopsy values for F (fluid volume) and pH (F) introduced earlier by Cheah et al. (1993, 1994, 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 as was discussed by Cheah et al., (1995). It appears that significant ($P < 0.05$) correlations were received between the parameters calculated from biopsy NMR measurements and the indicators of post mortem pH fall rate (pH 1h), conductivity (24 h), drip loss (48 h). By contrast, reflectance (630 nm) values were poorly related with biopsy NMR values (except ratio PCr + ATP/Pi + SP at 10 min). The correlations are lower if compare to earlier results received in halothane positive and negative pigs (Miri et al., 1989, Lahucky et al., 1993).

CONCLUSION

Our results 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. Inspite the presence of a single defective allele may not necessarily lead to drastic changes in the muscle metabolism, a consumer preferred meat quality can mainly be produced by directed breeding of homozygous NN-genotypes also in the fattening stage.

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Table 1 Evaluation of PCr/(Pi + SP) and (PCr + ATP)/(Pi + SP) in biopsies from heterozygous and normal pigs

	min	Nn				NN	
		pH < 6.0 (n = 7)		pH > 6.0 (n = 6)		(n = 7)	
		$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± 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}

Means with different superscripts are different at $P < 0.05$

Table 2 Ante-mortem pH biopsy and post-mortem meat quality traits of pigs with different RYR 1 gene status

		Nn				NN	
		pH < 6.0		pH > 6.0			
		$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Ante mortem pH		5.85	0.1^{a}	6.00	0.1^{a}	6.32	0.1^{b}
Post mortem							
pH (contact)	1h	5.83	0.1^{a}	6.15	0.1^{b}	6.36	0.1^{c}
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}
Reflectance (630 nm)		52.70	10.0	44.76	3.7	45.35	4.9

Means with different superscrips are different at $P < 0.05$