LIPOLYSIS DURING CHILLED STORAGE OF PORK

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Background. Pig breeds show extensive variation in the rate of post mortem glycolysis in relation with their HAL gene status, as the n allele induces glycolysis acceleration (see Sellier and Monin, 1994, for review). The glycolysis rate affects meat quality through protein denaturation, particularly water holding capacity (Bendall and Wismer-Pedersen, 1962) and tenderness (Buchter and Zeuthen, 1971). Much less is known of the effects on lipids. Rate of glycolysis can be expected to influence activities of lipolytic enzymes as it is the case for a number of glycolytic enzymes (Talmant and Monin, 1983). Moreover, Cheah et al. (1986) have found an increased phospholipase A_2 activity in muscle of halothane-positive pigs. This enzyme is activated by sarcoplasmic calcium which is much higher in halothane-positive pigs (Cheah et al., 1984, 1986).

Objectives. The present experiment was aimed to investigate lipolytic changes in muscle from Large White (homozygous for the N allele of the HAL gene) and Piétrain pigs (homozygous for the n allele) during chilled storage. Phospholipase A_2 activities and sarcoplasmic calcium were determined as they could contribute to possible breed variation in lipolysis.

Material and methods.

Animals and sampling. Ten NN LW pigs and 6 nn Piétrain pigs were fattened till 100 kg liveweight when they were slaughtered in the laboratory abattoir. The day after slaughter, six chops of Longissimus lumborum were packed individually under vacuum and put in a chilling room at 3-4 °C. At 1, 5 and 9 days after slaughter, one chop was used for extraction of mitochondria and sarcoplasmic Ca++. Another chop was frozen in its package and kept at -35 °C for further lipid analysis.

Analytical techniques. Five g of minced muscle were homogenized in 25 ml KCl 0.15 M at 4 °C. The mixture was centrifuged at 1000 *g* for 10 min. The supernatant was filtrated then centrifuged at 8000 *g* for 10 min. The supernatant (sarcoplasmic fraction) was kept at -80 °C and the pellet homogenized again in 10 ml KCl 0.15 M and centrifuged for 10 mn. The pellet (mitochondrial fraction) was suspended in 2 ml (KCl 0.15 M, MOPS 10 mM, pH 7.4) then kept at -80 °C till analysis. For the determination of phospholipase A_2 activity, 2 ml of 0.075 M phosphate-0.015 M citrate buffer (pH 5.5 or 7) was added with 50 µl mitochondrial suspension, 250 µl 0.317 mM Ph-NBD and 150 µl (0.15 M KCl, MOPS 10 mM, pH 7.4). The mixture was incubated for 30 min at 37 °C, added with 500 µl 0.1 N HCl to stop the reaction, then extracted with 3 ml of TMBE. Fluorescence was read at λexc/em 465/535 nm.. Activity was calculated against a standard curve obtained by extracting aqueous solutions of NBD.C6 with TMBE (final concentration 0.05 to 0.5 ppm). Sarcoplasmic calcium was extracted according to Cheah et al.(1984) from 30 grams of muscle minced in 50 ml of 0.15 M KCl. After mineralization of 2 ml of supernatant, Ca was determined by atomic absorption.

Lipids were extracted according to Folch from 20 g of muscle homogenized in 250 ml of (CHCl3:MeOH, 2:1) then separated by passing 30-60 mg of lipid in 5 ml of (CHCl3:MeOH, 2:1) through an aminopropyl column. Glycerides were eluted with 5 ml of(CHCl3:isopropanol, 2:1), free fatty acids with 5 ml of (diethylic ether, acetic acid 2%), and phospholipids with 5 ml of (MeOH:HCl, 9:1). Fatty acids of each fraction were determined by gas chromatography after methylation according to Diaz (1994).

Statistics. Data were analyzed using repeated measurement variance analysis. Means comparison among chilling times was carried out by paired t-test.

Results and discussion. Large White pigs tended to have higher intramuscular fat than Piétrain pigs (1.8 % vs 1.4 %, $P > 0.05$). Free fatty acid content was higher in Piétrain than in Large Whites, the difference reaching significance at day 5 (Table 1). Total lipids of Large White pigs contained more saturated, more monounsaturated and less polyunsaturated fatty acids (Table 2). This was related to differences in fatty acid composition of glycerides. Phospholipids of Large White pigs contained slightly more monounsaturated fatty acids than those of Piétrain pigs. These breed differences could be related to the HAL gene status. In fact, Hartmann et al. (1992) reported that longissimus intramuscular fat contained more saturated and less polyunsaturated fatty acids in halothane-negative than in halothane-positive pigs. Chilling time affected markedly the level and the composition of free fatty acids (Table 1). There were interactions betwen time and genetic type on free fatty acid composition. The percentages of saturated and monoinsaturated fatty acids decreased and the percentage of polyinsaturated fatty acids increased with time in Large White pigs while they did not change significantly between day 1 and day 9 in Piétrain pigs. This indicated that lipolysis affected more the phospholipids than the glycerides in Large Whites.

Both genetic type and time affected sarcoplasmic calcium (Table 3). The latter was higher at day 1 in Piétrains than in Large Whites then the difference decreased. This breed difference was probably related to halothane status as established by Cheah et al. (1984). Phospholipase A2 was not significantly affected by either genetic type or chilling time, although there was a marked trend to a higher pH 7 activity in Large White pigs (Table 3). Moreover a trend to decrease in pH 5.5 activity was found between days 5 and 9 in Piétrain pigs. Our results are in contrast with those reported by Cheah et al. (1986) who found higher phospholipase A2 activity in Piétrain pigs. However, it must be noted that these authors measured phospholipase activity from samples obtained within 5 min after death. It can be assumed that activity decreased more in Piétrain pigs during the post mortem period due to denaturation by low pH-high temperature conditions. Such conditions are known to induce extensive protein denaturation and decrease in activity of some enzymes (Talmant and Monin, 1983).

Conclusions. Our results showed that changes affecting muscle lipids during chilled storage for 9 days differed between Large White and Piétrain pigs. They indicated that phospholipids were more affected in Large White pigs. This could be related to the trend to higher phospholipase A_2 activities observed in this breed.

References.

Bendall, J.R. and Wismer-Pedersen, J. (1962). J. Food Sci., 27, 144.

Buchter, L. and Zeuthen, P. (1971). Proceed. 2nd Int. Symp. Condition Meat Quality of Pigs, Zeist, 247.

Cheah, K.S., Cheah, A.M., Crosland, A.R. and Casey, J.C. (1984). Meat Sci., 10, 117.

Cheah, K.S., Cheah, A.M., Crosland, A.R. and Waring, J.C. (1986). Meat Sci., 17, 37.

Diaz, I. (1994). Thesis, Universitat Autonóma de Barcelona.

Hartmann, S., Otten, W., Kratzmair, M., Berrer, A. and Eichinger, H.M. (1992). 38th ICoMST, Clermont-Ferrand, 77.

Sellier, P. and Monin, G. (1994). J. Muscle Foods, 5, 187.

Talmant, A. and Monin, G. (1983). 29th Europ. Meet. Meat Res. Workers, 1, 27.

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Breed		Large Whites			Piétrains			SEM	Pt	Pb	Pt x Pb
Day		*day 1*	*day 5*	*day 9*	*day 1*	*day 5*	*day 9*				
FFA,	mg/g muscle	0.18^{a}	0.27^{b}	0.49^{c}	0.22^{a}	0.36^{b}	0.58^{c}	0.02	**	*	ns
FFA, %	Saturated	38.9^{a}	34.8^{b}	32.6^{b}	36.8^{a}	35.2^{b}	38.4^{ab}	0.6	**	ns	**
	MonoUnsat.	27.2^{a}	20.9^{b}	22.1^{b}	22.2	18.8	19.8	0.8	**	ns	ns
	PolyUnsat.	33.9^{a}	44.3^{b}	45.3^{b}	41.0^{a}	46.0^{b}	41.8^{ab}	1.0	**	ns	*

Table 1. Changes in content and composition of free fatty acid in Longissimus muscle from Large White and Piétrain pigs during chilled storage.
Pt : significance of time effect ; Pb : significance of breed effect ; * P< 0.05 ; ** P < 0.01. Results are expressed as means. Means from one breed within a row with a different superscript differ at P < 0.05 ; means underlined within a same column differ at P < 0.05.

Breed		Large Whites			Piétrains			SEM	Pt	Pb	Pt x Pb
Day		*day 1*	*day 5*	*day 9*	*day 1*	*day 5*	*day 9*				
Total lipids	Saturated	34.8^{a}	34.8^{a}	35.5^{b}	35.0^{a}	33.5^{b}	33.3^{ab}	0.2	**	*	*
	MonoUns.	36.2	36.6	38.4	35.2	34.0	34.1	0.5	ns	*	ns
	PolyUns.	29.0	28.6	26.2	31.8	32.6	32.5	0.6	ns	*	ns
Glycerides	Saturated	35.8	35.9	35.8	33.6	33.2	33.0	0.3	ns	**	ns
	MonoUns.	45.2	44.2	45.2	43.3	42.5	43.9	0.4	ns	ns	ns
	PolyUns.	19.0	19.9	18.0	23.1	24.2	23.1	0.6	ns	**	ns
Phospholipids	Saturated	33.7^{a}	35.8^{b}	34.2^{ab}	32.8^{a}	33.1^{ab}	36.3^{b}	1.3	ns	n	ns
	MonoUns.	14.2	13.3	13.6	11.0^{a}	11.7^{ab}	12.8^{b}	0.3	ns	**	*
	PolyUns.	52.1	50.9	52.2	56.3	55.2	50.9	1.5	ns	ns	ns

Table 2. Changes in fatty acid composition of total lipids, glycerides and phospholipids in Longissimus muscle from Large White and Piétrain pigs during chilled storage.
Pt : significance of time effect ; Pb : significance of breed effect * P< 0.05 ; ** P < 0.01. Results are expressed as means in percent. Means from one breed within a row with a different superscript differ at P < 0.05.

Breed	Large Whites			Piétrains		
Day	*day 1*	*day 5*	*day 9*	*day 1*	*day 5*	*day 9*
Sarcoplasmic Ca^{2+}, µg/g	0.49 ± 0.08	1.01 ± 0.21	1.22 ± 0.06	1.07 ± 0.07	1.32 ± 0.06	1.20 ± 0.09
Phospholipase A_2, pH 5.5	342 ± 114	396 ± 62	375 ± 100	329 ± 147	313 ± 215	90 ± 40
Phospholipase A_2, pH 7	293 ± 76	317 ± 73	200 ± 37	145 ± 58	128 ± 61	141 ± 86

Table 3. Sarcoplasmic Ca^{2+} and phospholipase A_2 activities in Longissimus muscle from Large White and Piétrain pigs during chilled storage.
Ca is expressed in µg/g muscle ; phospholipase is expressed in ng substrate/min/g muscle. Results are given as means ± sem.