

EFFECT OF PIG GENETIC TYPE ON MEAT ANTIOXIDANT, LIPOLYTIC AND PROTEOLYTIC ENZYMES ACTIVITIES.Hernández P.¹, Zomeño L.², Ariño B.², Blasco A.².¹ Departamento de Producción Animal y Ciencia y Tecnología de los Alimentos, Universidad Cardenal Herrera-CEU. 46113 Moncada, Spain.² Departamento de Ciencia Animal, Universidad Politécnica de Valencia. 46071 Valencia, Spain.**Background**

Many studies have reported the influence of pig breed and crossbreed on growth, carcass composition (Blasco et al., 1994) and meat quality (Oliver et al., 1993, 1994), but only few have studied the influence of genetic type on the enzymatic activity of pork meat. Oxidative processes in meat lead to meat quality deterioration. Meat has endogenous antioxidants and prooxidants. Information on factors influencing the activity of antioxidant enzymes, in meat is limited. Antioxidant enzymes activities differ between meat of different species (Pradhan et al., 2000) and muscle type (Hernández et al., 2000). Variations of the activity of these enzymes between different genetic types could lead to differences in oxidative stability of the meat.

Muscle lipases and phospholipases contribute to the hydrolysis of lipid fraction releasing free fatty acids and related by products (Hernández et al. 1999) and cathepsins are involved on structural and biochemical changes that take place during post-mortem storage of meat (Koochmaria 1988). Differences in the levels of the activity of these enzymes result in differences of sensory properties of pig meat.

Objectives

The objective of this study was to study the differences between five pig genetic types on the activity of antioxidant, lipolytic and proteolytic enzymes in meat.

Methods

Forty psoas mayor of females of five different pig genetic types were used in this experiment, Pietrain (P), Large-White (LW), Landrace (LR), Iberian (I), and Iberian x Duroc (I x D). At 24h post mortem the psoas major were dissected and frozen at -20°C until they were required for enzymatic analysis.

Assays of antioxidant enzyme activities. Catalase activity assay was performed as described by Aebi (1983). One unit (U) of catalase was defined as the amount of extract needed to decompose 1 µM of H₂O₂ per min. Glutathione peroxidase activity (GSH-Px) was determined by measuring the oxidation of NAPH (De Vore & Greene, 1982; Gunzler & Flohe, 1985). One unit of GSH-Px was defined as the amount of extract required to oxidize 1 µM of NADPH per min. Superoxide dismutase activity (SOD) was determined using the NADH oxidation method of Paoletti and Mocali (1990). One unit of SOD activity was defined as the amount of extract required to inhibit the rate of NADH oxidation by the control (no SOD) by 50%. All the antioxidant enzymes assays were performed at 25°C.

Assays of lipase activities. Acid lipase acid, acid phospholipase and neutral lipase were assay as previously described Motilva et al. (1992) using 4-methylumbelliferyl oleate as fluorescent substrate. Reaction mixture of lipases assays with fluorimetric substrates was incubated at 37°C for 20 min. The fluorescence was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. One unit of lipolytic activity is defined as the amount of enzyme capable of hydrolysing 1 µmol of substrate in 1 h at 37°C.

Assays of cathepsins activities. Cathepsin B, B+L and H were assayed as previously described by Toldrá and Etherington (1988), using N-CBZ-L-arginyl-7-amido-4-methylcoumarin, N-CBZ-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin, both a pH 6.0, and L-arginine-7-amido-4-methylcoumarin at pH 6.8 as specific fluorimetric substrates of cathepsin B, B+L and H, respectively. The reaction mixtures with fluorimetric substrates were incubated at 37°C and fluorescence was continuously at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. One unit of cathepsin activity was defined as the amount of enzyme hydrolysing 1 µmol of substrate per minute at 37°C.

Data were analysed using the general Linear Model (GLM) Procedure of SAS (1996) program.

Results and discussion

Differences between genetic types were found for the activity of catalase and SOD, while GSH-Px showed no differences with the genetic types (table 1). The highest differences between genetic types were found for the Iberian and Iberian X Duroc breeds when compared with the White pigs. Besides, catalase activity showed differences between the White pigs, with large values for LR and lower activities in P and LW. No effect of carcass weight was found for any of the enzymes. Previous studies have indicated that endogenous antioxidants enzymes, especially catalase, could potentially delay the onset of oxidative rancidity in stored meat (Mei et al. 1994; Pradhan et al. 2000). The differences between genetic types found in the enzyme activity suggest some genetic determination for the antioxidant activity of meat. Very little is known about the post-mortem activities of lipolytic enzymes in skeletal muscles. Table 2 shows the values of lipolytic activities measured in pork meat from different genetic types. There were no differences for acid and neutral lipase activities between the different genetic types. The activity of acid phospholipase showed small differences being higher the activity in P and lower in LR. Armero et al. (1999) found small differences for acid lipase activity when studied the influence of the terminal sire type, showing the Belgium Landrace lower activity than the Danish Duroc.

Differences were found between proteolytic enzymes activities between the genetic types studied (table 3). Iberian pigs (I) had the lowest values for cathepsin B and H. These results agree with those reported by Rosell & Toldrá (1998). The higher levels of cathepsins in White pigs could be related to their earlier slaughtering age (6 month vs 12 month) since the protein turnover decrease with animal age (Goll 1991). Differences between the White pigs were found on the cathepsin activities, with lower values for LR when compared with P and LW.

Pertinent literature

- Aebi H. E. 1983. *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, Vol 3, pp. 273-286. Verlag Chemie, Weinheim, Germany.
 Armero E., Barbosa J. A., Toldrá F., Baselga M., Pla M. 1999. *Meat Sci.* 51: 185-189.
 Blasco A., Gou P., Gispert M., Estany J., Soler Q., Diestre A., Tibau J. 1994. *Liv. Prod. Sci.* 40: 171-178.
 De Vore V. R., Greene B. E. 1982. *J. Food Sci.* 47: 1406-1409.

- Goll D. E. 1991. Muscle Bioch. Reciprocal Meat Conf. Proc. 44: 25-36.
- Gunzler A., Flohe L. 1985. CRC handbook of methods for oxygen radical research. ed. R. A. Greenwald, Vol 1, pp. 285-290. Boca Raton, Florida, USA.
- Hernández P., Navarro J. L., Toldrá F. 1999. Z Lebensm Unters Forsch 208: 110-115.
- Hernández P., Park D. K., Rhee K. S. 2000. IFT Congress. Dallas, TX. USA.
- Koohmaraie M., Babiker A. S., Merkel R. A., Dutson T. R. 1988. J. Food Sci. 5: 1253-1257.
- Mei L., Crum A. D., Decker E. A. 1994. J. Food Lipids. 1: 273-283.
- Motilva M-J., Toldrá F., Flores J. 1992. Z Lebensm Unters Forsch 195:446-450.
- Oliver M. A., Gispert M., Diestre A. 1993. Meat Sci. 35:105-118.
- Oliver M. A., Gou P., Gispert M., Diestre A., Arnau J., Noguera J. L., Blasco A. 1994. Liv. Prod. Sci. 40: 179-185.
- Paoletti F., Mocali A. 1990. Methods In Enzymology. 186: 209-219.
- Pradhan A., A., Rhee K. S., Hernández P. 2000. Meat Sci. 54:385-390.
- Rosell C. M., Toldrá F. 1998. J. Sci. Food Agric. 76: 117-122.
- SAS. 1996. SAS Institute Inc. Cary, NC.
- Toldrá F., Etherington D J. 1988. Meat Sci. 23: 1-7.

Table 1. Antioxidant enzymes activities of meat from different pig genetic types

(U/g)	P		LW		LR		I x D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Catalase	187 ^a	18	217 ^a	17	294 ^b	17	324 ^{bc}	24	381 ^c	24
GSH-Px	0.177	0.022	0.199	0.021	0.176	0.021	0.210	0.029	0.227	0.029
SOD	657 ^a	39	636 ^a	37	637 ^a	37	928 ^b	53	739 ^a	53

LSM: least square means. SE: standard error. Means with different superscript, within a raw, differ significantly, P<0.05.

Table 2. Lipase activities of meat from different pig genetic types

(U/g)	P		LW		LR		I x D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Acid Lipase	0.197	0.011	0.207	0.009	0.191	0.007	0.194	0.010	0.221	0.010
Neutral Lipase	2.18	0.38	1.91	0.31	1.94	0.24	2.45	0.34	1.98	0.34
Phospholipase	0.111 ^c	0.009	0.079 ^{ab}	0.007	0.078 ^a	0.006	0.102 ^{bc}	0.008	0.101 ^{bc}	0.009

LSM: least square means. SE: standard error. Means with different superscript, within a raw, differ significantly, P<0.05.

Table 3. Cathepsin activities of meat from different pig genetic types

(U/g)	P		LW		LR		I x D		I	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
B	0.747 ^c	0.078	0.639 ^c	0.064	0.470 ^{ab}	0.050	0.558 ^{bc}	0.070	0.349 ^a	0.070
B + L	2.46	0.28	2.26	0.23	2.16	0.18	2.45	0.25	1.88	0.25
H	1.18 ^c	0.09	1.11 ^{bc}	0.07	0.948 ^{ab}	0.057	1.19 ^c	0.08	0.766 ^a	0.081

LSM: least square means. SE: standard error. Means with different superscript, within a raw, differ significantly, P<0.05.