PAPER

VARIATION IN MEAT QUALITY CHARACTERISTICS BETWEEN LANDRACE AND SICILIAN PIGS

V. ALFEO[1], S. VELOTTO[2], S. DE CAMILLIS[2], T. STASI[3] and A. TODARO*[1]

[1]Department of Agricultural, Food and Forest Sciences, Università degli Studi di Palermo, Viale delle Scienze ed. 4, Palermo, Italy

[2]Department of Promotion of human sciences and the quality of life, University of Study of Roma San Raffaele, Via di Val Cannuta 247, Roma, Italy

[3]Department of Science and Technology, Newton Consulting srl, Italy

*Corresponding author: aldo.todaro@unipa.it

ABSTRACT

This study compared the meat quality of Sicilian and Landrace pigs breeds and supported these results with biochemical and histological measurements on the samples collected from the m. *Longissimus Dorsi*, at the level of the 8th thoracic vertebra, before electrical stimulation. Twenty clinically healthy swine, 10 male (5 for each pig breed) and 10 female (5 for each pig breed), were slaughtered at 1 year of age at a body mass of 135±10 kg and 150±10 respectively for Sicilian and Landrace pigs. Particularly on the muscle considered the morphometric characteristics of FG (*fast glycolytic)*, FOG (*fast oxidative glycolytic*) and SO (*slow oxidative*) fibre types and their percentage were determined. Measurements related to myofibrillar fragmentation, sarcomere length and connective tissue properties gave convincing support. Sicilian pig produced more tender meat than Landrace, mainly due to favourable calpain-to-calpastatin ratios.

Keywords: connective tissue, meat quality, proteolytic system, sarcomere length

1. INTRODUCTION

Most recently, autochthonous breeds have been recognized as important elements to regional agro-biodiversity and, more specifically, in their relevance to agro-ecosystems that encompass the cultural heritage of a given region (JOVANOVI *et al*. 2011). It is relevant the role that local breeds could play to the regional development of the countries, including social, environmental and economic sustainability issues in a globalized market (BELIBASAKI *et al*. 2012). Furthermore, there has been an increasing interests in local breed because of the high quality perceived by consumers of meat and meat products from indigenous breed (STANIŠIĆ *et al*., 2015).

Other researchers investigated the characteristics, in term of meet quality, of local pigs breed by comparing to purebred lines and their crossbreed (DEBRECÉNI *et al.,* 2016, STANIŠIĆ *et al*., 2015; POTO *et al.,* 2007; MORCUENDE *et al.,* 2007) and no relevant literature was found about Sicilian pig breed. This last pig breed is also called Nebrodi or Madonie black swine. The interest in indigenous pig breeds is related to meat products. Particularly among them, the Sicilian pig is widely appreciated for the high quality of its niche products linked to the local gastronomic traditions (VELOTTO *et al.,* 2007). Precocious and long-lived breed it is characterized by a peculiar fertility and a great number of weaned piglets. This breed is also resistant to diseases and to the inclemency of the weather, being able to appreciate poor food as well. Italian Landrace has become dominant as an improved breed in Italy. They are long pigs with pink skin, white hair and ears that droop over their eyes. The original stock was brought to Italy from Scandinavian sources, but selection has been toward meat characteristics. Landrace pigs are a common second breed in crossbreeding programs in modern production, because of their good meat structure and maternal abilities (VELOTTO *et al.,* 2014). They are very efficient at converting food to meat, and produce little excess fat. Landraces are known for their rapid growth when young, typically reaching a heavier weight at weaning than other breeds, which is another factor in their lasting popularity in factory farms. Physical and chemical changes occur during storage of meat after slaughtering that affect the meat quality. In particular, tenderness and the mechanism of meat tenderization represent one the most debated subject among the scientific community.

Several researchers investigated the mechanism of meat tenderization and factors responsible for the initiation and course of this process (KOOHMARAIE, 1996; ROWE *et al*., 2004; LI *et al*., 2012). CHRIKI *et al*. (2012) showed that increased meat tenderness is related lower insoluble collagen and total collagen content, lower cross-sectional area of fibres, and an overall fibre type composition displaying more oxidative fibres than glycolytic fibres. MOHRHAUSER *et al*. (2014) showed that the decrease in m-calpain in the delay-chilled bovine carcasses could explain the improvements of the myofibrillar fragmentation index postmortem. The myofibrillar fragment length (MFL) could represents an indicator of meat tenderness depended on specific proteolytic enzyme activity, such as m-calpain. The activity of μ- and m-calpain is synergistic: μ-calpain contributes to early post-mortem proteolysis, while m-calpain is partially activated and contributes to tenderization during prolonged ageing (VARRICCHIO *et al*., 2013).

In this study variation in meat tenderness between Landrace and Sicilian pig breeds were investigated. Particularly, supportive histological and biochemical evidence was provided to explain the variation in these quality characteristics. The aim of this work was to compare the Landrace breed with Sicilian pig. The study focused attention on size and distribution of fibre types of *Longissimus Dorsi* (LD), one of the most studied muscles.

2. MATERIAL AND METHODS

2.1. Slaughter and sampling procedures

Muscle samples were collected from pigs of the pure autochthonous breed "Sicilian black swine" and the breed "Italian Landrace" slaughtered in an abattoir. Animals were reared in an extensive system and raised using the most traditional farming methods, the diet of swine shifted gradually from woodland forage and scraps to corn. Twenty clinically healthy swine (10 male and 10 female: 5 male and 5 female Landrace; 5 male and 5 female Sicilian pig) from farms located in south and north Italy, were slaughtered at 1 year of age at a body mass of 135±10 kg and 150±10 respectively for Sicilian and Landrace pigs. Carcasses were electrically stimulated for 60 s after exsanguinations (400 V peak, 5ms pulses at 15 pulses/s) and entered the cold rooms 45 min after killing. Animals handling followed the recommendations of the European Community (Reg. CE 1/2005; directives 74/577/EEC; Law 439 2 August 1978) concerning animal care. Samples for muscle fibre typing were collected from the *m. Longissimus Dorsi* (LD), at the level of the 8th thoracic vertebra, before electrical stimulation. Samples were collected for measurements of sarcomere length, muscle fibre type, collagen properties, and proteolytic enzyme activities. Sarcomere length was performed on fresh samples (2, 9 and 16 days post mortem). Samples for the remaining tests were vacuum packaged and frozen in liquid nitrogen (-196°C) and stored at -80°C.
For each of the following methods the tests were performed in triplicate.

2.2. Muscle biochemistry

Samples collected for enzyme studies (24 h post mortem) were frozen in liquid nitrogen and preserved at -80°C. Calpastatin in combination with m-calpain and m-calpain was extracted from 5 g of the LD frozen samples as described by DRANSFIELD (1996) and separated by means of the two-step gradient ion-exchange chromatography method according to GEESINK and KOOHMARAIE (1999). Calpain assays were determined by using azocasein as substrate according to DRANSFIELD (1996). Data were expressed as units/g of muscle. Frozen samples of the LD were analysed for collagen content and solubility according to the method described by BERGMAN and LOXLEY (1963), HILL (1966) and WEBER (1973).

2.3. Histological measurements

Samples for sarcomere lengths of fresh LD samples (24 h post mortem), were prepared according to the method of HEGARTY and NAUDÈ (1970) by using distilled water instead of Ringer – Locke solution (DREYER *et al.*, 1979). Myofibrils were extracted according to CULLER *et al.* (1978) as modified by HEINZE and BRUGGEMANN (1994). The sections were stained histochemically for myosin ATPase (myosin ATPase reveals muscular contraction) and succinic dehydrogenase (SDH reveals metabolism) simultaneously on the same myofibres (SOLOMON and DUNN 1988; VELOTTO *et al.*, 2010).

2.4. Statistical analyses

The data were subjected to analysis of variance (Gen Stat - RVSN International, Hemel Hempstead, UK; PAYNE *et al*., 2007). Treatment means were separated using Fisher's protected t-test least significant difference at the 5% level of significance.

3. RESULTS AND DISCUSSION

Variation in MFL in the two pigs showed that Sicilian pig exhibited lower MFL compared to Landrace pig (Table 1).

Table 1. Breed comparison for histological measurements.

Myofibrillar fragment length (µm)	Sicilian pig	Landrace
2 days of aging	33.9^A	41.4^B
9 days of aging	28.1^A	34.4^B
16 days of aging	28.1^A	28.3^A
2 vs. 16 days	10.8^A	13.2^B
Sarcomere length (µm)	1.57^A	1.92^B

Different letters in the row indicate significant level of p<0.001

Particularly at 16 days post mortem, the differences among breeds were not significant, whereas the changes in MFL between 2 and 9 days post mortem were higher for Landrace samples indicating a higher degree of fragmentation over the length of aging. Landrace had longer sarcomere lengths than Sicilian pig (p<0.001). The fibre type composition was delineated histochemically in pig muscle samples, identifying three fibre types: FG, with high myosin ATPase activity and low oxidative activity; FOG, with moderate myosin ATPase activity and intermediate oxidative activity; and SO, with low myosin ATPase and high oxidative activity. The differences between Landrace and Sicilian pigs for the LD muscle, with regard to fibre area were 18% for FG, 18% for FOG and 22% for SO. Sicilian pig had the smallest fibre area for all fibre types and differed significantly from Landrace (Table 2).

Regarding the histological features of muscle LD, it is confirmed that is a light muscle due to the higher percentage of FG both in Landrace than Sicilian pigs (Figs. 1 and 2).

The proteolytic enzyme activity was analysed at two days post-mortem and gave the following results: landrace had higher calpastatin activity and calpastatin: m-calpain ratio and lower m-calpain activity than Sicilian pig (p<0.001; Table 3), Sicilian pig had a non-significantly higher m-calpain than Landrace pig.

About collagen content were found some differences among breeds, particularly Sicilian pig had less total collagen and soluble collagen (p<0.001) than Landrace.

Most of the tenderness-related results in this study clearly distinguish Sicilian pig from Landrace breed. It is important to know the physical and chemical changes that occur during storage of meat after slaughtering to develop a classification system for both the tenderness of the meat rather than optimize the same tenderness process. The importance of µ-calpain for tenderization process was reported by numerous authors (KOOHMARAIE, 1996; ROWE *et al*. 2004; LI *et al*. 2012). The mechanism of meat tenderization is complicated. Numerous investigations are being conducted which are aimed at explaining the mechanism of meat tenderization and factors responsible for the initiation and course of this process. The activity of µ- and m-calpain is synergistic: µ-calpain contributes to early post-mortem proteolysis, while m-calpain is partially activated and contributes to tenderization during prolonged ageing (VARRICCHIO *et al*. 2013). In this study, favourable lower calpastatin (inhibitor) and higher calpain activities characterized the Sicilian pig, the lower activity of m-calpain in Sicilian pig revealed a probably activation of this enzyme in prolonged ageing. Variation in MFL among breeds, especially at 2 days post mortem, corresponded with the variation in proteolytic enzyme activity showing less fragmentation for Landrace pig in early post mortem compared with the Sicilian one. The relatively higher rate of fragmentation (measured as differences between 2 and 16 days MFL) for Landrace pig breed with prolonged aging suggested delayed aging for the tougher breeds.

Table 2. Mean value and coefficient of variation (cv,%) of morphometric characteristics of fibre types in the considered muscles.

	Fibre Types					
	Sicilian pig					
Muscle*	**FG[a]**		**FOG[a]**		**SO[a]**	
	mean	**v.c.,%**	**mean**	**v.c.,%**	**mean**	**v.c.,%**
	Area/μm^2					
LDb	5400^{A}	36	3803.80^{A}	37	6058.42^{A}	36
	Percentage (%)					
LDb	45		35		20	
	Landrace					
Muscle*	**FG[a]**		**FOG[a]**		**SO[a]**	
	mean	**v.c.,%**	**mean**	**v.c.,%**	**mean**	**v.c.,%**
	Area/μm^2					
LDb	6600^{B}	44	4649.09^{B}	45	7404.73^{B}	44
	Percentage (%)					
LDb	48		35		17	

[a]SO: red; FOG: intermediate; FG: white. [b]LD = m. *Longissimus Dorsi*. Different letters in the column indicate significant level of $p<0.001$ (A vs. B)

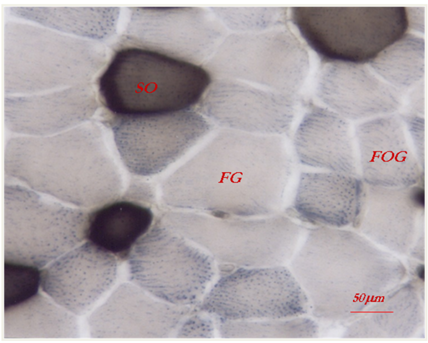

Figure 1. *Longissimus Dorsi* muscle Sicilian Pig. FOG = fast oxidative glycolytic, FG = fast glycolytic, SO = slow oxidative.

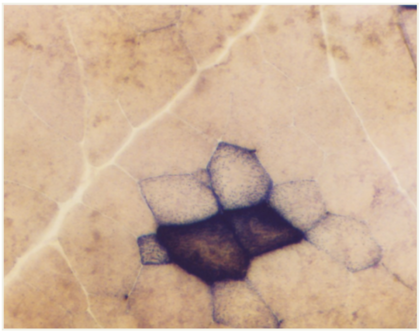

Figure 2. *Longissimus Dorsi* muscle. Landrace.

Table 3. Breed comparison for biochemical characteristics of *m. Longissimus Dorsi.*

Proteolytic enzyme activity	**Sicilian**	**Landrace**
Calpastatin [a]	4.3^{B}	5.3^{A}
m-calpain [b]	1.2^{A}	0.9^{A}
m-calpain [b]	0.8^{A}	0.9^{A}
Calpastatin: m-calpain ratio	4.11^{B}	5.02^{A}
Calpastatin: m-calpain ratio	5.49^{B}	6.72^{A}
Calpastatin: total calpain ratio	2.08^{B}	2.29^{A}
Connective tissue properties		
Total collagen (mg/g)	3.55^{B}	4.34^{A}
Soluble collagen (%)	18.8^{B}	22.9^{A}

[a]Calpastatin activity: one unit of calpastatin is defined as the amount that inhibited one unit of m-calpain activity.

[b]One unit of calpain activity is defined as an increase in absorbance at 366 nm of 1.0 absorbance unit per gram of muscle per hour at 25°C. Different letters in the row indicate significant level of $p<0.001$.

The activity of m-calpain was highly correlated with that of calpastatin and MFL and this finding supports the hypothesis that m-calpain initiates the breakdown of myofibril proteins. The negative correlation between m-calpain and MFL indicated that fewer proteolysis occurred in muscle when myofibril particles are large, and therefore, MFL as a determinable indicator of meat tenderness depended on activities of m-calpain. The same conclusion was found by MOHRHAUSER *et al.* (2014) according to which in their study on bovine the decrease in μ-calpain activity in the delay chilled carcasses could explain the improvements in myofibrillar fragmentation index postmortem. Several studies have demonstrated that muscle fibre type composition contributes to the variation in meat quality (VELOTTO *et al*. 2014). However, according to Table 2, the fibre characteristics of Sicilian pig and Landrace presented some differences as confirmed by their tenderness measurements supported by proteolytic enzyme activities that showed differences in favour of Sicilian pig. With the histochemical staining procedure used in this study three muscle fiber types (SO, FOG, and FG) can be differentiated. The ratio of slow-twitch oxidative (type I) and fast-twitch glycolytic (type II) muscle fibers also evidently influence meat tenderness. The ratio varies among individual animals of the same breed, breeds, and crosses. CHRIKI *et al*. (2012) in their results showed that increased meat tenderness is related lower insoluble collagen and total collagen content, lower cross-sectional area of fibres, and an overall fibre type composition displaying more oxidative fibres than glycolytic fibres. It is possible to identify light and dark muscles in the pig. Particularly our study shows that LD muscle had a high percentage of FG fibre types in both species. Muscles harboring a higher percentage of type IIB fibers tend to result in a generally poorer meat quality than muscles harboring a higher percentage of type I fibers (CHOI *et al*. 2013). But it is possible to notice the presence of more developed SO fibres in LD in both breeds. Landrace sarcomere lengths were significantly shorter ($p<0.05$) than that of Sicilian pig. Tenderness is also positively related to the sarcomere length. Within sarcomeres, the contractile filaments actin and myosin act together in the cross-bridge cycle, determining the state of contraction post mortem. In this study sarcomere length could have contributed to the slightly tougher meat of the Landrace together with other factors discussed previously. Sicilian pig had significantly less soluble collagen than Landrace ($p<0.05$). According to WOJTYSIAK (2013) the decrease in soluble collagen content during growth of pigs are important factors influencing shear force value, and thus raw meat tenderness. Therefore, other factors also accounted for the variation in tenderness. Variation in colour and water-binding characteristics can be related to variation in the glycolysis rate and muscle temperature decline.

In conclusion, the parameters analysed provided the best explanation for variation in meat tenderness and particularly it seems that the Sicilian pig has a slight disposition in terms of tenderness when compared with Landrace. Meat quality is the result of a multi-factor process which begins from the moment of slaughter and to a large extent depends on the breed and species of the animal from which the meat comes.

REFERENCES

Belibasaki S., Sossidou E. and Gavojdian D. 2012. Local Breeds: Can they be a competitive Solution for Regional Development in the World of 'Globalization'? The Cases of Greek and Romanian Local Breeds. Animal Science and Biotechnologies 45:278-284.

Bergman I. and Loxley R. 1963. Two improved and simplified methods for the spectrophotometric determination of Hydroxyproline. Analytical Chemistry 35:1961-1965.

Choi M., Nam K.W., Choe J.H., Ryu Y.C., Wick M.P., Lee K and Kim B.C. 2013. Growth, carcass, fiber type, and meat quality characteristics in Large White pigs with different live weights. Livestock Science 155:123-129.

Chriki S., Gardner G.E., Jurie C., Picard B., Micol D., Brun J.P., Journaux L. and Hocquette J.F. 2012. Cluster analysis application identifies muscle characteristics of importance for beef tenderness. BMC Biochemistry 13:29.

Culler R.D., Parrish F.C., Smith G.C. and Cross H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine *Longissimus* muscle. Journal Of Food Science 43:1177-1180.

Debrecéni O., Komovà P. and Bučko O. 2016. Comparison the physicochemical quality indicators of Musculus *Longissimus* Dorsi from Mangalitsa breed and their crossbreeds. Journal of Central European Agriculture 17(4):1253-1263.

Dransfield E. 1996. Calpains From Thaw Rigor Muscle. Meat Science 43:311-320.

Dreyer J.H., Van Rensburg A.J.J., Nade' R.T., Gouws P.J. and Stiemie S. 1979. The effect of chilling temperatures and mode of suspension of beef carcasses on sarcomere length and meat tenderness. South African Journal of Animal Science 9:1-9.

Solomon M.B. and Dunn M.C. 1988. Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine and porcine skeletal muscle. Journal of Animal Science 66(1): 55-264.

Geesink, G.H. and Koohmaraie, M. 1999. Effect of calpastatin on degradation of myofibrillar proteins by μ-calpain under postmortem conditions. Journal of Animal Science 77:2685-2692.

Hegarty P.V.J. and Naudè R.T. 1970. The accuracy of measurement of individual skeletal muscle fibres separated by a rapid technique. Laboratory Practice 19:161-163.

Heinze P.H. and Bruggemann D. 1994. Ageing of beef: Influence of two ageing methods on sensory properties and myofibrillar proteins. Sci. Aliment. 14:387-391.

Hill F. 1966. The solubility of intramuscular collagen on meat animals of various ages. Journal of Food Science 31:161-166.

Jovanovi S., Savi M., Aleksi S. and Živkovi D. 2011. Production standard s and the quality of milk and meat products from cattle and sheep raised in sustainable production systems. Biotechnology in Animal Husbandry 27:397-404.

Koohmaraie M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. Meat Science 431: 193-201.

Li C.B., Li J., Zhou G.H., Lametsch R., Ertbjerg P., Brüggemann D.A., Huang H.G., Karlsson A.H., Hviid M. and Lundström K. 2012. Electrical stimulation affects metabolic enzyme phosphorylation, protease activation, and meat tenderization in beef. Journal of Animal Science 90:1638-49.

Mohrhauser D.A., Lonergan S.M., Huff-Lonergan E., Underwood K.R. and Weaver A.D. 2014. Calpain-1 activity in bovine muscle is primarily influenced by temperature, not pH decline. Journal of Animal Science 92:1261-1270.

Morcuende D., Estévez M., Ramìrez R. and Cava R. 2007. Effect of the Iberian x Duroc reciprocal cross on productive parameters, meat quality and lipogenic enzyme activities. Meat Science 76:86-94.

Poto A., Galiàn M. and Peinado B. 2007. Chato Murciano pig and its creosses with Iberian and large White pigs, reared outdoors. Comparative study of the carcass and meat characteristics. Livestock Science 111:96-103.

Rowe L.J., Maddock K.R., Lonergan S.M. and Huff-Lonergan E. 2004. Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. Journal of Animal Science 82(11):3254-3266.

Solomon M.B. and Dunn M.C. 1988. Simultaneous histochemical determination of three fibre types in single section of ovine, bovine and porcine skeletal muscle. Journal of Animal Science 66:255-264.

Stanišić N., Radović Č., Stajić S., Živković D. and Tomašević I. 2015. Physicochemical properties of meat from Mangalista pig breed. Meso 17(1):50-53.

Weber R. 1973. The Determination of hydroxyproline and chloride in meat and meat products: simultaneous operation with nitrogen and phosphorus determinations. Technical Report 7, Technicon International Division Sa, Geneva.

Wojtysiak D. 2013. Effect of age on structural properties of intramuscular connective tissue, muscle fibre, collagen content and meat tenderness in pig *longissimus lumborum muscle*. Folia Biologica (Krakow) 61:221-6.

Varricchio E,. Russolillo M.G., Maruccio L., Velotto S., Campanile G., Paolucci M. and Russo F. 2013. Immunological detection of m- and - calpains in the skeletal muscle of Marchigiana cattle. European Journal of Histochemistry 57:e2.

Velotto S., Varricchio E., Di Prisco M.R., Stasi T. and Crasto A. 2007. Skeletal myocyte types and vascularity in the black sicilian pig. Acta Veterinaria Brno 76:163-170.

Velotto S., Vitale C., Stasi T. and Crasto A. 2010. New insights into muscle fibre types in casertana pig. Acta Veterinaria Brno 79:169-176.

Velotto S., Vitale C., Varricchio E. And Crasto A. 2014. A new perspective: an Italian autochthonous pig and its muscle and fat tissue characteristics. Indian Journal of Animal Research 48:143-149.

Paper Received November 27, 2018 Accepted April 10, 2019