



Relationship between Myosin Isoforms and Meat Quality Traits in Pig Semitendinosus Neuromuscular Compartments

Guillermo H Graziotti*, José M Rodríguez Menéndez, Clara M Ríos, María E Cossu¹, Alexis Bosco, Néstor O Affricano, Alejandra Paltenghi Ceschel, Sonia Moisés¹ and Lorenzo Basso¹

Anatomy Area, Veterinary School, Buenos Aires University, Chorroarín 280, CABA, CWO 1427, Argentine

ABSTRACT : The aim was to determine the relationship between muscle structure and meat quality traits in neuromuscular compartments (NMCs: R1, R2, R3, R4) of pig semitendinosus muscle. Barrows from the INTA-MGC genetic line (Argentina) were slaughtered at 100 kg body weight. In each NMC the following parameters were determined: the fibre types I, IIA, IIX and IIB by immunohistochemistry, the fibre cross sectional area (FCSA), the pH of meat after 24 h post-mortem (pH₂₄), instrumental meat tenderness (WB) and colour (L*, a*, b*). There were significant differences in the following: L* (R1 = R4 < R2 = R3), a* (R1 > R4 > R2 = R3), b* (R1 = R4 < R2 = R3), WB (R2 > R1 = R3 = R4), pH₂₄ (R1 = R4 > R2 = R3). The relative percentages of FCSA were as follows: I (R4 > R1 > R3 > R2), IIA (R1 > R4 > R3 > R2), IIX (R1 = R2 = R3 = R4) and IIB (R2 > R3 > R1 > R4). The correlation values were statistically significant between IIB and WB (R1 and R4, r_s = 0.66), (R2 and R3 r_s = 0.74), IIB and L* (R1 and R4 r_s = 0.84), IIX and L* without discriminating NMCs. Our data suggest that the NMC where the sampling takes place is important for determining meat quality traits because of the heterogeneity of the whole muscle. (**Key Words** : Anatomy, Neuromuscular Compartment, Fibre Muscle, Meat Quality, Pig)

INTRODUCTION

Meat quality can be evaluated by several traits, such as pH₂₄, the instrumental meat tenderness by Warner-Bratzler shear force (WB) and colour (L*, a* and b*). Intrinsic muscle characteristics, such as fibre types, the fibre cross sectional areas (FCSA), perimortem metabolism, and environmental components including feeding and rearing system have an impact on the change from muscle to meat by modifying the values of the meat quality traits (Karlsson et al., 1999; Park et al., 2007; Nam et al., 2009). Furthermore, it is important to note that mammalian skeletal muscles are not homogeneous. Mammalian skeletal muscles are composed of neuromuscular compartments (NMCs) defined by the distribution of primary nerve branches that may alter the muscle fibre characteristics and the metabolic profile (Roy et al., 1984; Bruce et al., 1993; Kernell, 1998; Graziotti et al., 2004; Graziotti et al., 2009). The myosin

heavy chain (MyHC) isoforms, I, IIA, IIX and IIB (with decreasing oxidative capacity in that order), are expressed in pig skeletal muscles (Quiroz-Rothe and Rivero, 2004) and vary significantly among different NMCs (Graziotti et al., 2008). The relationship between muscle fibre types and meat quality in pigs is inconsistent and not completely understood (Gentry et al., 2004) probably for not to keep in mind the muscular partitioning in heterogeneous NMCs. In this study the aim was to investigate in each NMC the relationship between the fibre characteristics and meat quality traits.

MATERIALS AND METHODS

Gross anatomy

To identify the NMCs, in a previous assay (Graziotti et al., 2009) the left semitendinosus (ST) muscle was removed from five carcasses preserving the sciatic nerve branches. The muscles were cleaned of fat and connective tissues, immersed in a 10% formalin solution for 30 days, rinsed with tap water for 3 days and incubated with a 25% nitric acid solution for 10 days while checking the muscles every 48 h during the entire procedure. Macroscopic dissections

* Corresponding Author : Guillermo H Graziotti. Tel: +54-011-45 24-8460, Fax: +54-011-4524-8480, E-mail: ggrazio@fvvet.uba.ar

¹ Meat Research Institute, Agronomy School, Buenos Aires University, Av. San Martín 4453, CABA, CWO 1417, Argentine.

Received June 29, 2010; Accepted September 6, 2010

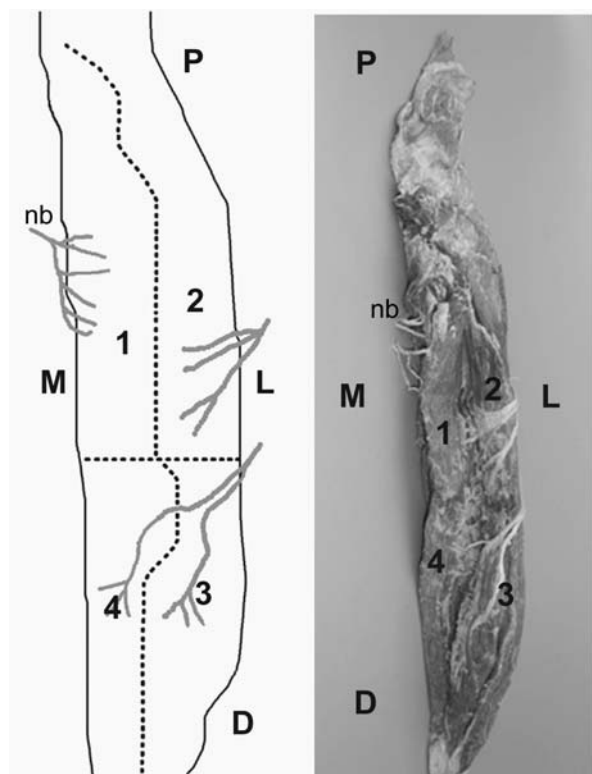


Figure 1. Scheme and ST muscle (cranial view) after treatment with nitric acid showing the NMCs: R1 (1), R2 (2), R3 (3) and R4 (4); nb indicates primary muscle branches of the sciatic nerve; P, proximal; D, distal; M, medial; L, lateral.

were carried out following the primary branches of the sciatic nerve (Figure 1).

Immunohistochemistry

This research was carried out on 12 right ST muscles of barrows ($n = 12$) from the INTA-MGC genetic line (Argentina) slaughtered at 100 kg body weight. Muscle samples for immunohistochemistry were taken from the core of each NMC (R1, R2, R3 and R4) 3 h postmortem, and the samples were frozen by immersion in liquid nitrogen and kept at -80°C until analysis. To identify the fibre types, $10\ \mu\text{m}$ thick serial sections were obtained using a cryostat at -27°C , and the sections were then mounted on glass slides and reacted by adenosine triphosphatase myofibrillar (mATPase) after acid preincubation (pH 4.6) (Brooke and Kaiser, 1970) modified by Nwoye et al. (1982). Other serial sections were incubated with a panel of

monoclonal antibodies (MAbs) that were specific for MyHC isoforms (Table 1). The immunohistochemical procedure with the avidin-biotin peroxidase complex (ABC) was used for the localisation of primary antibody binding as described Graziotti et al. (2004). In brief, sections were preincubated in a blocking solution of stock goat serum. After the primary antibody was diluted in Phosphate Buffered Saline, the antibody was applied to the sections for 40 min in a humid chamber at 37°C . The sections were then washed and incubated with a secondary antibody. After incubation with the secondary antibody, the sections were washed and incubated with the ABC reagent. Diaminobenzidine tetrahydrochloride was used as a chromogen to localise the peroxidase. The proportion of hybrid fibres was irrelevant ($<3.5\%$), but the immunohistochemically delineated fibre types were characterised as the pure fibre types (I, IIA, IIX and IIB) according to Abreu et al. (2006).

For quantitative studies the FCSA were determined by captured images in a TIFF format employing the Motic Image Plus 2.0 software. The FCSA of each individual fibre type was determined in the slides treated with mATPase with an image ($10\times$ magnification) by drawing a mask along the cell borders (100 fibres at least). Opensource software (Scion Image beta 3b) was utilised for determining the FCSA.

Meat quality traits

The entire 12 right ST muscles ($n = 12$) used for immunohistochemistry were chilled and the NMCs (R1, R2, R3 and R4) were sampled 24 h postslaughter to determine the pH_{24} (pHmeter Testo 230), colour by values of brightness L^* , redness (a^*) and yellowness (b^*) (colorimeter Minolta CR-300) (CIE, 1976) and WB after a 50 min incubation in a water bath (75°C) (Instron 4442).

Statistical analysis

Statistical analysis was carried out by Friedman's test, being the NMCs the treatments and the animals the blocks and $p < 0.05$ was considered statistically significant. The Spearman's test was used for the correlation analysis.

RESULTS

The fibre types have been identified by

Table 1. Specificity of MAbs against adult skeletal MyHC isoforms used in this study and immunohistochemical characterization of pure skeletal muscle fibre types in pig (I, IIA, IIX and IIB types) according to the MyHC isoform they express

MAbs	Dilution	I	IIa	IIX	IIB	References
BA-F8	1:10	+	-	-	-	Graziotti et al., 2004
A4.74	1:10	-	+	\pm	-	Graziotti et al., 2001
BF-35	1:10	+	+	-	-	Graziotti et al., 2001
A4.1519	1:10	-	+	+	-	Graziotti et al., 2001

immunohistochemistry and histochemistry as follows: type I (MAb BA-F8+; mATPase dark), type IIA (MAb A4.74+; mATPase light), type IIX (MAbs A4.1519+, BF-35-; mATPase intermediate) and type IIB (MAb A4.1519-; mATPase intermediate) as shown in Figure 2 and Table 1.

The percentage of fibre type sum areas as follows: type I ($R4 > R1 > R3 > R2$), type IIA ($R1 > R4 > R3 > R2$), type IIB ($R2 > R3 > R1 > R4$) and type IIX ($R1 = R2 = R3 = R4$) as shown in Table 3.

There were significant differences ($p < 0.05$) among the NMCs with regard to colour in L^* ($R1 = R4 < R2 = R3$), a^* ($R4 > R1 > R2 = R3$) and b^* ($R1 = R4 < R2 = R3$). The R2 compartment had significant differences in the WB values when compared to the other three NMCs ($R2 > R1 = R3 = R4$). The pH values were significantly different among all four NMCs ($R1 = R4 > R2 = R3$).

The correlation between the fibre type IIB and the WB

value was statistically significant with regard to the R1 and R4 NMCs ($r_s = 0.66$) as well as the R2 and R3 NMCs ($r_s = 0.74$). The correlation between the fibre type IIB and L^* value was statistically significant with regard to R1 and R4 ($r_s = 0.84$). The correlation between the fibre type IIX and L^* value was statistically significant without discriminating NMCs.

DISCUSSION

The ST muscle, a strongly partitioned muscle, was used for this study because it has the phenotypic expression of the I, IIA, IIB and IIX MyHC isoforms (Lefaucheur, 2006).

In accordance with Park et al. (2007) our results indicate that the MyHC isoforms, expressed in each of the studied NMCs, are relevant attributes in meat quality development because they influence perimortem metabolism. The meat

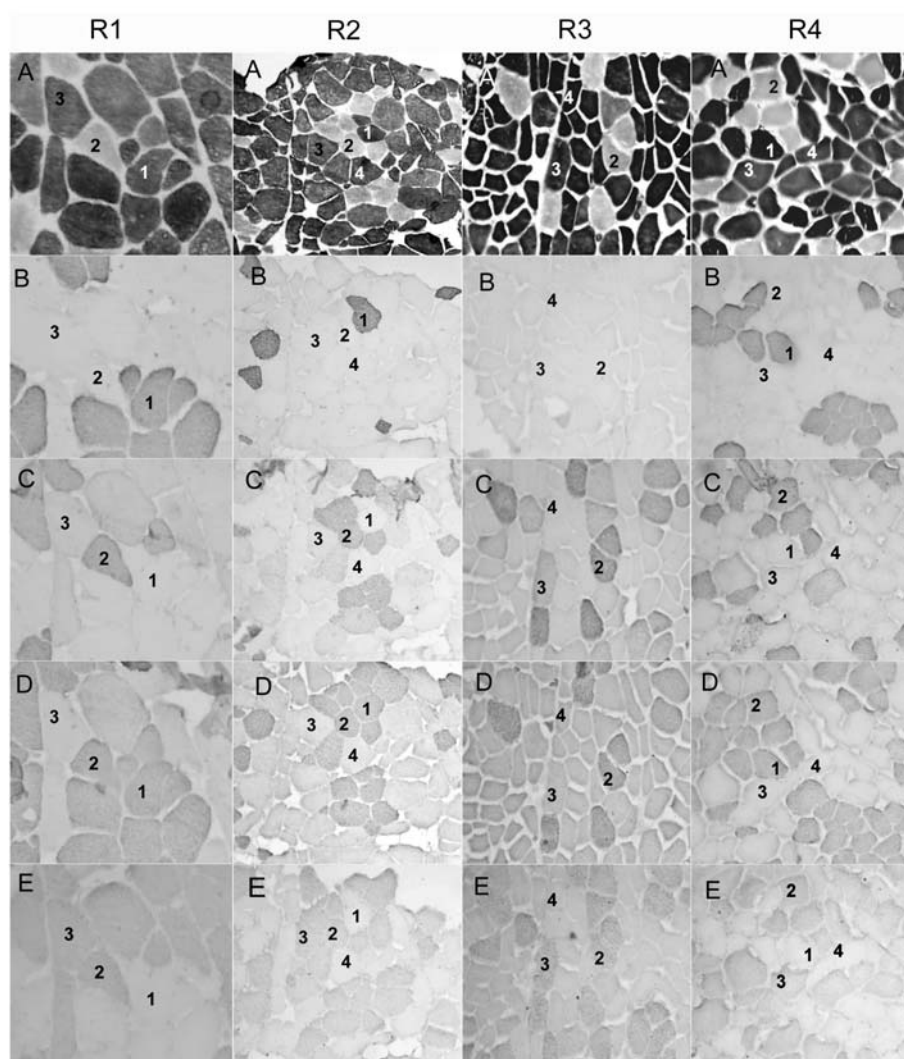


Figure 2. Serial sections of the NMCs (R1, R2, R3 and R4) stained for mATPase activity after acid preincubation (pH 4.6). (A), MAb BA-F8 (B), MAb A4.74 (C), MAb BF-35 (D), and MAb A4.1519 (E); 1, 2, 3 and 4 correspond to fibre type I, IIA, IIX and IIB respectively. Scale 100 \times .

Table 2. Meat quality traits values (L*, a*, b*, WB and pH₂₄) according to the NMCs (R1, R2, R3 and R4)

	R1	R2	R3	R4
L*	70.23 ^a (2.7)	80.63 ^b (4.39)	84.42 ^b (4.09)	70.07 ^a (3.54)
a*	18.48 ^a (1.70)	9.28 ^b (1.71)	9.33 ^b (2.01)	24.58 ^c (14.94)
b*	11.79 ^a (2.13)	19.33 ^b (1.84)	19.96 ^b (1.621)	13.06 ^a (2.80)
WB	7.85 ^a (1.77)	9.49 ^b (2.35)	8.25 ^a (1.42)	7.97 ^a (1.59)
pH ₂₄	6.11 ^a (0.27)	5.91 ^b (0.30)	5.97 ^b (0.24)	6.14 ^a (0.24)

Within a row those values with the same letter do not present significant differences ($p > 0.05$).

The standard deviation (SD) values are indicated between brackets ($n = 12$).

quality trait values in each of the oxidative NMCs (R1 and R4) and glycolytic NMCs (R2 and R3) correspond to the fibre muscle characteristics in glycolytic or oxidative muscles that were determined in prior investigations (Gentry et al., 2004; Hu et al., 2008; Gil et al., 2008).

The higher a* values and the lower L* and b* values in the R1 and R4 NMCs are in agreement with a relationship among a* values, red fibre percentages (I-IIa MyHC isoforms) and oxyhaemoglobin values. (Park et al., 2007; Kwasiborski et al., 2008; Gil et al., 2008; Bérard et al., 2008; Nam et al., 2009).

Such as red fibres contain higher myoglobin values, the pH values decline slowly. This may explain the greater pH₂₄ values in the R1 and R4 NMCs when compared to the R2 and R3 NMCs (Kwasiborski et al., 2008). Similarly, the smaller values found in the R2 and R3 NMCs may be explained by the greater rate and longer period of postmortem glycolysis in these two NMCs (Bee et al., 2006).

Although the relation between the WB values and muscle fibre characteristics is unclear (Sazili et al., 2005), a positive correlation among the WB values, FCSA and percentage of type IIB fibres was suggested (Hu et al., 2008; Nam et al., 2009). In accordance, the results in this study indicated that the FCSA of the type IIB (Table 3) and the WB value (Table 2) were significantly higher in the R2

NMC when compared to the other NMCs.

According to Gentry et al. (2004) muscular structure in animal husbandry research is not completely understood. Some authors (Cerisuelo et al., 2007; Hu et al., 2008) use numerous sampling places to correct variations due to the muscle heterogeneity (Algañaraz, 2007). The heterogeneity among muscle NMCs requires accurate anatomical references for muscle sampling because the conclusions based on a single muscle sampling location are not representative (Janz et al., 2006; Lefaucheur, 2006). A standard sampling following neuromuscular partitioning may be more appropriate because previous muscular architecture studies (Graziotti et al., 2009) indicate that NMCs are composed of muscular fibres with homogeneous characteristics.

We conclude that sampling according to the distribution of primary nerve branches while maintaining the three-dimensional orientation and the relation with anatomical references is an appropriate tool for examining the entire muscle.

ACKNOWLEDGMENTS

This research was supplied by Grant V-803 UBACYT, Buenos Aires University, Argentina.

The monoclonal antibodies A4.74 and A4.1519

Table 3. Percent of fibre FCSA

Fibre types	R1 (%)	R2 (%)	R3 (%)	R4 (%)
I	31.69 ^a (12.45)	3.68 ^b (3.28)	5.89 ^c (6.93)	35.82 ^d (5.98)
IIA	28.17 ^a (5.74)	11.81 ^b (8.01)	16.45 ^c (9.48)	23.82 ^d (4.79)
IIX	30.85 ^a (11.59)	31.34 ^a (6.57)	39.44 ^a (13.35)	32.31 ^a (6.43)
IIB	9.27 ^a (10.30)	53.16 ^b (12.43)	39.02 ^c (16.87)	6.66 ^d (7.88)

Within a row those values of each variable with the same letter do not present significant differences ($p > 0.05$).

The standard deviation (SD) values are indicated between brackets ($n = 12$).

developed by Helen M Blau, BA-F8 and BF-35 developed by Stefano Schiaffino were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242, USA.

REFERENCES

- Abreu, E., E. Quiroz-Rothe, A. I. Mayoral, J. M. Vivo, A. Robina, M. T. Guillén, E. Agüera and J. L. L. Rivero. 2006. Myosin heavy chain fibre types and fibre sizes in nuliparous and primiparous ovariectomized Iberian sows: interaction with two alternative rearing systems during the fattening period. *Meat Sci.* 74:359-372.
- Algañaraz, L. 2007. Predicción de la purga (exudado) de carne de cerdo (*Sus scrofa domestica*), en bandeja, basada en las características de la canal. Thesis. Zamorano University, Honduras.
- Bee, G., C. Biolley, G. Guex, W. Herzog, S. M. Lonergan and E. Huff-Lonergan. 2006. Effects of available dietary carbohydrate and preslaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. *J. Anim. Sci.* 84:191-203.
- Bérard, J., M. Kreuzer and G. Bee. 2008. Effect of litter size and birth weight on growth, carcass and pork quality and their relationship to postmortem proteolysis. *J. Anim. Sci.* 86:2357-2368.
- Brooke, M. M. and K. K. Kaiser. 1970. Muscle fiber types: how many and what kind? *Arch Neurol.* 23:369-379.
- Bruce, V. L., R. J. Turek and W. A. Schurg. 1993. Muscle fibre compartmentalization in the gluteus medius of the horse. *Equine Vet. J.* 25:69-72.
- Cerisuelo, A., R. Sala, G. Nürnberg, M. Baucells and C. Rehfeldt. 2007. How many muscle samples are required to obtain reliable estimations of muscle fibre characteristics from pig longissimus muscle? *Meat Sci.* 76:583-587.
- CIE. 1976. Commission International de l'Eclairage, 18th Session, 1975. CIE Publication 36, Paris.
- Gentry, J. G., J. J. McC Glone, M. F. Miller and J. R. Blanton. 2004. Environmental effects on pig performance, meat quality and muscle characteristics. *J. Anim. Sci.* 82:209-217.
- Gil, M., M. I. Delday, M. Gispert, M. Fonti Furnols, C. M. Maltin, G. S. Plastow, R. Klont, A. A. Sosnicki and D. Carrión. 2008. Relationships between biochemical characteristics and meat quality of Longissimus thoracis and Semimembranosus muscles in five porcine lines. *Meat Sci.* 80:927-933.
- Graziotti, G. H., G. Delhon, C. M. Ríos and J. L. L. Rivero. 2001. Fiber muscle types in adult females pigs as determined by combining histochemical and immunohistochemical methods. *Rev. Chil. Anat.* 19:167-173.
- Graziotti, G. H., P. Palencia, G. Delhon and J. L. L. Rivero. 2004. Neuromuscular partitioning, architectural design and myosin fiber types of the muscle vastus lateralis of the llama (*Lama glama*). *J. Morphol.* 262:667-681.
- Graziotti, G. H., C. M. Ríos, J. M. Rodríguez Menéndez, M. Salinas, A. Paltenghi Ceschel, A. Bosco, N. O. Affricano, C. Victorica and L. Basso. 2008. Capacidad metabólica del músculo de cerdo en sistemas confinado/semiextensivo. Estudio preliminar. *Rev. Arg. Prod. Anim.* 28:111-117.
- Graziotti, G. H., C. M. Ríos, J. M. Rodríguez Menéndez, M. Salinas, A. Bosco, A. Paltenghi Ceschel, N. O. Affricano and C. L. Victorica. 2009. Muscular partitioning in the semitendinosus muscle of the pig. *Int. J. Morphol.* 27:947-953.
- Hu, H., J. Wang, R. Zhu, J. Guo and Y. Wu. 2008. Effect of myosin heavy chain composition of muscles of meat quality in Laiwu pigs and Duroc. *Sci. China Series C: Life Sci.* 51:127-132.
- Janz, J. A. M., J. L. Aalhus, M. E. R. Dugan and M. A. Price. 2006. A mapping method for the description of Warner-Bratzler shear force gradients in beef Longissimus thoracis et lumborum and Semitendinosus. *Meat Sci.* 72:79-90.
- Karlsson, A. H., R. R. Klont and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork quality. *Livest. Prod. Sci.* 60:255-269.
- Kernell, D. 1998. Muscle regionalization. *Can. J. Appl. Physiol.* 23:1-22.
- Kwasiborski, A., T. Sayd, C. Chambon, V. Santé-Lhoutellier, D. Rocha and C. Terlouw. 2008. Pig Longissimus lumborum proteome: Part II: Relationships between protein content and meat quality. *Meat Sci.* 80:982-996.
- Lefaucheur, L. 2006. Myofibre typing and its relationships to growth performance and meat quality. *Arch. Tierz.* 49 (Special Issue): 4-17.
- Nam, Y. J., Y. M. Choi, S. H. Lee, J. H. Choe, D. W. Jeong, Y. Y. Kim and B. C. Kim. 2009. Sensory evaluations of porcine longissimus dorsi muscle: Relationships with postmortem meat quality traits and muscles fiber characteristics. *Meat Sci.* 83:731-736.
- Nwoye, L., W. F. H. M. Mommaerts, D. R. Simpson, K. Sreyderian and M. Marusich. 1982. Evidence for a direct action of thyroid hormone in specifying muscle properties. *Am. J. Physiol. Cell Physiol.* 242:R401-R408.
- Park, B. Y., N. K. Kim, C. S. Lee and Y. H. Hwang. 2007. Effect of fiber type on postmortem proteolysis in longissimus muscle of Landrace and Korean native black pigs. *Meat Sci.* 77:482-491.
- Quiroz-Rothe, E. and J. L. L. Rivero. 2004. Coordinated expression of myosin heavy chains, metabolic enzymes, and morphological features of porcine skeletal muscle fiber types. *Microsc. Res. Tech.* 65:43-61.
- Roy, R. R., P. L. Powell, P. Kanim and D. R. Simpson. 1984. Architectural and histochemical analysis of the semitendinosus muscle in mice, rats, guinea pigs, and rabbits. *J. Morphol.* 181:155-160.
- Sazili, A. Q., T. Parr, P. L. Sensky, S. W. Jones, R. G. Barsley and P. J. Buttery. 2005. The relationship between slow and fast myosin heavy chain content, calpastatin and meat tenderness in different ovine skeletal muscles. *Meat Sci.* 69:17-25.