Influence of Genotype and Diet on the Characteristics of *Semitendinosus* Muscle in Crossbred Young Bulls Derived from Brown Swiss Cow and Double Muscled Bulls

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Summary

The aim of this study was to evaluate the influence of genotype and diet on the characteristics of muscle fibers and adipocytes of the *semitendinosus* muscle in crossbred young bulls derived from Brown Swiss cows (B) and double-muscled Piemontese (PI) or Belgian Blue (BB) bulls. For this purpose 24 young bulls divided in 6 groups fed 3 diets have been used: a control diet without supplementation of rumen protected CLA (rpCLA), two other diets added with 8 or 80 g/d of a supplement of rpCLA. The histochemical methods (succinic dehydrogenase and mATPase) pointed out the presence of three fiber types: type I, type IIA and type IIX. Results demonstrated that genotype affected both fiber type number and size. While there was no significant difference among the percentages of type I fibers (PI×B 12.73%, BB×B 12.95%), the difference was significant ($P<0.05$) for IIA fiber type (PI×B 23.03%, BB×B 29.13%) and for IIX fiber type (PI×B 64.53%, BB×B 57.84%). All the fiber types of the BB×B genotype had a significantly bigger size ($P<0.05$) than the fibers of PI×B genotype. The adipocytes analyses showed a significant effect ($P<0.05$) of genotype on the adipocytes total surface. The level of CLA addition did not affected neither muscle fiber nor adipocytes characteristics.

Key words

bulls, genotype, *semitendinosus* muscle, muscle fiber characteristics, CLA

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Aim

The aim of this study was to evaluate the influence of the genetic type and of the rumen protected conjugates linoleic acid (rpCLA) supplementation on the characteristics of muscle fibers of the *semitendinosus* muscle, and on the number and dimensions of the adipocytes in crossbred young bulls derived Brown Swiss cow (B) sired with double-muscled bulls (PI or BB).

Material and methods

The project was approved by the "Ethical Committee for the care and the use of experimental animals" of the University of Padua, and is part of a research aimed at the study of low protein diets and rpCLA addition (Schiavon et al., 2010, 2011, 2012) and nutrient requirements (Schiavon and Bittante, 2012) of purebred and crossbred dual purpose young bulls. The study comprised a total of 24 crossbred young bulls from Brown Swiss cow (B) sired with double-muscled bulls (PI or BB). Animals were divided in two groups according to genotypes, 12 belonging to BB×B and 12 belonging to PI×B genotype. Each genotype was divided into 3 subgroups fed with 3 diets: a control diet (Schiavon et al., 2011) without supplementation of rpCLA, or with 8 g/d or 80 g/d of a rpCLA supplement. The animals were slaughtered all together at the age of 18 months, with a live weight of kg 662±30 (B×BB) and kg 624±53 (PI×B).

Histochemical analysis - About two hours after slaughter, 24 samples of *semitendinosus* muscle *(pars superficialis)* were collected from the left hind quarter, frozen in isopentane cooled by liquid nitrogen and stored at -80° until cutting. Pieces of frozen muscle samples were mounted on cryostat chuck, equilibrated at -25°C and cut perpendicularly to the muscle axis. Serial sections (10 μm thick) were stained using haematoxylin/eosin staining; myosin ATPase method (Brooke and Kaiser, 1970) was performed after acid pre-incubation (pH 4.30, 4.35, 4.40) and alkaline pre-incubation (pH 10.40, 10.44). Determination of succinate dehydrogenase activity (SDH) was also performed (Pearse, 1972). The coloration with Oil red (Bonucci, 1981) allowed to highlight the adipocytes located in *perimysium*.

Image analysis - Images were acquired and analysed with a semiautomatic image analysis system (Cell^B Soft Imaging Solutions, Olympus 2007). The following parameters were computed: percent number of different fiber types, cross sectional area (CSA) of each fiber type (μm^2) and percentage of area occupied by the different fiber types. An average of 250-300 muscle fibers/sample from two random fields was analysed. The surface area of single adipocytes was measured on four fields (total surface of 6.17×10^6 μm^2) and then summed. The connectival surface of *perimysium* on which adipocytes were located, was measured and summed.

Statistical analyses - The data were analysed statistically with the GLM procedure of SAS package (SAS Institute Inc., 1990) using a linear type III model considering genotype (G), diet (D) and their interaction (G×D).

Results and discussion

Meat producers attempt to offer quality meat to satisfy consumers' requests. Meat tenderness is the major sensory characteristic affecting meat quality and consumer acceptance (Huffman et al., 1996; Miller et al., 2001). It depends mainly on connective tissue content, structure and solubility. As regard fiber type composition Maltin et al. (1998) and Hwang et al. (2010) reported that increasing the proportion of slow-twitch type I fibers improved tenderness in cattle. Moreover muscle with a larger fiber size, exhibited tougher meat than muscles of smaller fiber size (Renand, 2001). However, in the cattle, the relationship between muscle fiber type and meat tenderness is still controversial (Maltin et al., 2003). The histochemical methods pointed out the presence of 3 fiber types: type I, type IIA and type IIX (fig. 1, 2). Only in two bulls a little percentage of fibers with intermediate mATPase activity was found; these fibers might correspond to hybrid fiber.

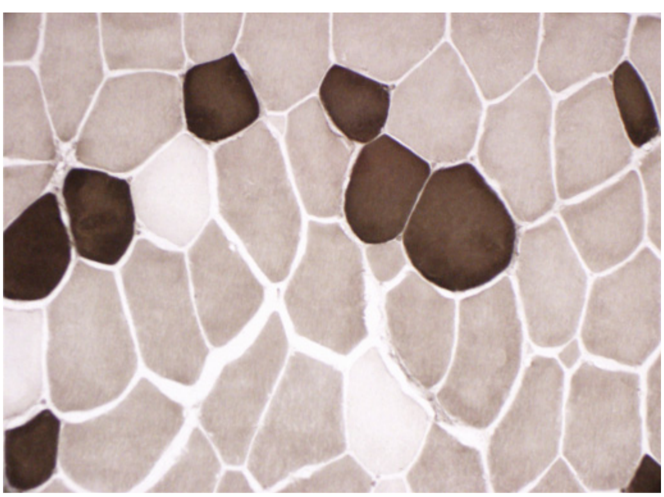

Figure 1. Cross-section of *semitendinosus* muscle from 18-mo-old Brown Swiss × Piemontese bull. Histochemical staining of fiber types with ATPase pH 4.40. Type I black, type IIA white, type IIX grey

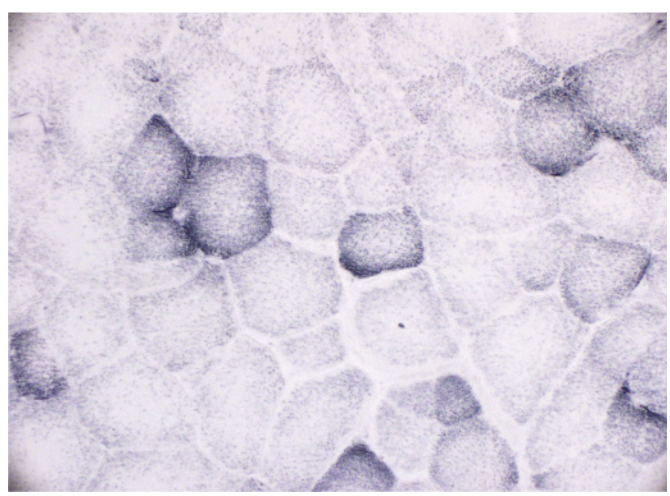

Figure 2. Cross-section of *semitendinosus* muscle from 18-mo-old Brown Swiss × Piemontese bull. SDH staining

Table 1. LSM of the effects of paternal breed, CLA supplementation level and relative interaction on fiber type composition as number proportion, cross sectional area and fiber area proportion

	Fiber number proportion (%)			Fiber cross sectional area (μm²)			Fiber area proportion (%)		
	I	IIA	IIX	I	IIA	IIX	I	IIA	IIX
Paternal breed:									
Belgian Blue(BB)	12.95	29.13*	57.84*	3472*	3433*	4374*	8.31	20.56**	51.29**
Piemontese (PI)	12.73	23.03*	64.53*	2588*	2924*	3934*	7.43	15.45**	58.17**
CLA supplied (g/d):									
0	12.45	28.41	59.61	2757	2989	4098	7.15	19.06	52.56
8	11.09	25.28	63.60	3372	3089	4041	7.21	17.17	56.80
80	14.98	24.55	60.33	2961	3458	4322	9.25	17.78	54.53
Residual rMSE[1]	4.79	5.82	5.66	919.8	583.0	565.8	3.31	4.97	5.42

* p<0.05, ** p<0.01; [1]rMSE = Root of mean square error.

Table 2. LSM of the effects of paternal breed, CLA supplementation level and relative interaction on adipocytes number, total and mean adipocytes area, and on *perimysium* surface

	N° adipocytes/field	Total area[1] (μm²)	Mean adipocyte area (μm²)	*Perimysium* surface (%)
Paternal breed:				
Belgian Blue (BB)	0.42	3960*	1410	3.03
Piemontese (PI)	0.48	6392*	3199	3.47
CLA supplied (g/d):				
0	0.59	4439	2181	3.55
8	0.37	4983	2287	2.84
80	0.37	6106	2445	3.37
Residual rMSE[2]	0.9	5922	2882	3.38

[1] area occupied from adipocytes on a total surface of $6.17 \cdot 10^6$ μm²; * p<0.05, ** p<0.01; [2]rMSE = root of mean square error.

Double-muscled breeds, Piemontese (PI) and Belgian Blue (BB) are known for their excellent lean growth performances (Albera et al., 2001). Double-muscled cattle produce meat of exceptional quality (Fiems et al., 1998; Boukha et al., 2011; Cecchinato et al., 2011a) being leaner and more tender and characterized by a lesser content of connective tissue (Wheeler et al., 2001; Alberti et al., 2008; Wiener et al., 2009), compared to cattle of conventional breeds, and they are used to produce crossbreds (Dal Zotto et al., 2009) with improved carcass and meat quality.

Results concerning analyses of variance on muscle fiber types and fiber size (CSA) of *semitendinosus* muscle in the different groups obtained in the present study are reported in Table 1. The *semitendinosus* muscle in its superficial part is a muscle characterized by a clear prevalence of rapid glycolytic fibres (Totland and Kryvi, 1991).

Comparing the genotypes of the bulls, there was no difference about the percentage of fibers Type I (PI×B 12.73%, BB×B 12.95%), while the difference was significant ($P<0.05$) for IIA fiber type (PI×B 23.03%, BB×B 29.13%) and for IIX fiber type (PI×B 64.53%, BB×B 57.84%). As for the dimensions of the fibers, results evidenced that all types of fibers of the genotype BB×B have a significantly bigger size ($P<0.05$) than those of PI×B. These results agree with data reported by Lazzaroni and Pagano (2003) in a morphometrical and histochemical comparative study in purebreed of the same two double-muscled breeds tested in current experiment as paternal breed. On average, fiber type IIX CSA found on the crossbred young bulls of the present study resulted smaller with respect to those found for the corresponding purebreeds (Wegner et al. 2000), whereas a CSA bigger than in purebreeds was measured in fiber type both IIA and I.

From the results of the present study, it can be speculated that the muscle thickness of the PI breed would be a consequence of fiber hyperplasia, while in the case of BB it seems more dependent on muscle hypertrophy; this difference could be related to the different mutation of the myostatin gene that characterize the two double-muscled breeds (Kambadur et al., 1997; McPherron and Lee, 1997) and could cause different characteristics of the meat produced by the two breeds.

Another important determinant of beef consumers satisfaction is flavour (Felderhoff et al., 2007). There seems to be a positive relationship between intramuscular fat (IMF) and palatability of meat (Wheeler et al., 1996). Intramuscular fat has a most important role on juiciness and flavour, and on cooking quality; IMF indirectly affects meat tenderness (Wood et al., 1999), and it is often very low in meats produced by double-muscled cattle (Cecchinato et al., 2011b).

Results obtained from the study of adipocytes are given in Table 2. Results underlined that the adipocytes total surface measured in the two paternal genotypes was significantly different ($P<0.05$) (PI×B 6392 μm², BB×B 3960 μm²). Also in the case of adipocytes the two paternal breeds differed in terms of number and size of the cells, quite the opposite observed for the muscle fibers.

Conclusion

We can conclude asserting that the genotype affected muscle fiber type and fibers dimension. The profile of *semitendinosus* muscle in the PI×B crossbred evidences a markedly glycolytic character, and this aspect could have consequences with regard to the progress of the aging and also to the color of the meat. The smaller dimensions of the fibers in the genotype PI×B compared to BB×B evidence that PI×B has thinner grained meat and likely a better tenderness. Genotype also affected adipocytes total surface which resulted more abundant in PI×B compared to BB×B. CLA supplementation seems not to influence the number and size of the different muscle fibers type and of the adipocytes.

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