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Muscle fiber properties in cattle and their relationships with meat qualities: an overview

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Abstract

The control of meat quality traits constitutes an important target for any farm animal production, including cattle. Therefore, a better understanding of the biochemical properties that drive muscle development and final outcomes, constitute one of the main challenging topics of animal production and meat science. Accordingly, this review focuses on skeletal muscle fibers in cattle and their relationships with beef qualities. It aimed to describe the chemical and structural properties of muscle fibers as well as a comprehensive review of their contractile and metabolic characteristics during the life of the animal. The existing methods for the classification of muscle fibers were reviewed, compared and discussed. Then, the different stages of myogenesis in cattle were defined. The main factors regulating fetal and postnatal growth and the plasticity of muscle fibers were evidenced especially the role of myostatin (MSTN) growth factor and the impact of nutritional factors. This review highlights that the knowledge about muscle fibers is paramount for a better understanding of how to control the muscle properties throughout the life of the animal for better management of the final eating qualities of beef. Accordingly, the associations between bovine muscle fibers and different meat eating qualities such as tenderness, pH decline and color traits were further presented.

Keywords: Skeletal muscle; Cattle; Myogenesis; Myosin heavy chains; Meat quality; Contractile and metabolic properties.

Introduction

Skeletal muscles represent the largest organ in the body that is composed of fibers which are long cylindrical polynucleated cells, organized in bundles that are surrounded by connective tissue. They are distributed over 75 to 90% of muscle volume. For a long time, the different muscles are distinguished based on their appearance or color, into white or red, and also according to their contractile properties into slow or fast.[1] From the large literature, earlier investigations on muscle fiber heterogeneity targeted on two specific diversities that are the metabolism and the contractile response, which are known to differ depending on the type of the muscle and the species.[2] In livestock species, the relationships between muscle properties and meat qualities were largely described in the three decades.[3-14] These properties are acquired during fetal life and evolved during all the life of animal.[15-18]

In this context, this review makes an attempt to cover the aspects related to muscle fibers and their plasticity according to several factors of variability such as the sex of the animals, their age, breed (*e.g.,* Continental *versus* British), the physiological status and the rearing practices or factors. Furthermore, the relationships between muscle fibers and several beef quality traits such pH decline, color and tenderness were reviewed.

The preparation of the database that served to write this comprehensive review was based on a computerized search using different databases including Google Scholar, Web of Science and Scopus. This allowed the identification of the major papers from the literature in addition to those from our group dealing with muscle fibers in cattle. Different keywords were used that are “muscle or muscle properties or muscle characteristics”, “fibre or fiber”, “myosins or myosin heavy chains”, “cattle or beef or bovine”, “meat or beef”, “meat or beef quality”; “color”, “tenderness or texture or Warner-Bratzler Shear force” and “color or colour”. The literature search focused exclusively on full text articles published in peer-reviewed journals.

Muscle fiber properties

Structure

Skeletal muscle consists of 74% water, 18% proteins, 4 – 5 % of lipids, 1% of carbohydrates and 1% of other substances such as vitamins mainly of group B, zinc, selenium and iron. These amounts may vary between species, breeds, part of the muscle in the carcass, etc. The individual muscle fibers are mostly composed of different types of proteins that are

categorized into contractile, cytoskeletal, sarcoplasmic and regulatory. They are composed of myofibrils distributed over the whole area of the fibers with a highly organized ultrastructure that is alternated by repeated structures known as sarcomeres.[6] Each sarcomere is delimited by Z-discs.[4] It is worthy to note that the sarcomere structure has fundamental effects on meat quality (for review:[19]).

The point of attachment of the Z-discs to the sarcolemma is at the level of structures called costamers, mainly via the desmin. Z-discs are highly organized three-dimensional structure composed of several well-known proteins such as nebulin, actin, titin, cap-Z, and α-actinin, but also of several other proteins that were recently reported and discovered namely using OMICs techniques, including myopalladin, telethonin and myotilin. A complete list of the of Z-disc proteins and their interaction has been described by Faulkner, et al. [20] In particular, myotilin was reported to play a pivotal role in the maintenance of muscle integrity.[21] Other proteins are present in the Z-discs but also extend into other part of the sarcomere or the sarcolemma, such as titin, nebulin and filamins. Z-discs are key elements in the architectural organization of the sarcomeric unit of striated muscle cells. It serves as a site coordinating various cellular signaling pathways mainly during myogenesis and for the control of muscle contraction. The ordered structure of each sarcomere as observed under electronic microscope was described to be an alignment of filaments that are thick and thin, and this latter interpenetrate the former. The thick and thin filaments are respectively composed mainly of myosin and actin. Other proteins constitute the thick filaments such as myosin binding proteins C and H (MYBPC and MYBPH) with specific Uniprot IDs of A6QP89 and Q0VBZ1, respectively, myomesin M protein and titin. The thin filaments had different composition and mainly contain nebulin, the complex of three distinct troponins that are troponin T, troponin C and troponin I, the archetypal-coiled coil of tropomyosin and some regulatory proteins[22]. All these proteins were reported to play an important role in muscle contraction, in the integrity of the sarcomere, and are further involved in cell signaling [21] as well as in meat quality variation of several traits.[23-26] Among these proteins, myosins that are a large and diverse family of proteins that all contain three primary elements represent 50% of the myofibrillar proteins. One molecule of myosin contains of 2 heavy chains and 4 light chains[27].

Contractile properties

Myosin heavy chain isoforms (MyHC) play a role in muscle contraction as they have an ATPase activity providing energy for muscle contraction and the MyLCs play a regulatory role. MyHC isoforms are usually used for the characterization of the contractile properties of fibers. Mammalian MyHCs are known to be coded by 11 different MYH genes that are highly conserved in vertebrate evolution.[27] The MYH genes are grouped in two clusters, the first one is composed of slow MyHC I/beta cardiac (*MYH7* gene) and α-cardiac (*MYH6* gene). The second cluster grouped the 3 fast isoforms MyHC IIa, IIx, IIb that are respectively coded by *MYH* 2, *MYH1* and *MYH4* genes (**Table 1**) and, two developmental isoforms expressed in embryonic and neonatal muscles: MyHC emb and neo coded respectively by the *MYH3* and *MYH8* genes.[22] The four isoforms I, IIa, IIx and IIb are predominant in adult skeletal muscles of different species (**Table 1**). MyHC I isoform is expressed in type I fibers that are slow, and the MyHC IIa, IIx and IIb fast isoforms are expressed respectively in the fibers IIA, IIX and IIB. The first studies in this field, reported that in mammals, the fast isoforms MyHC IIa and IIb were expressed respectively in the IIA and IIB fibers. However, the recent developments namely in the techniques of the identification of the types of myosins fibers allowed the identification of a third fast MyHC IIx isoform in several species including cattle, rat, human, and pig.[27] It is worthy to note that the fast MyHC IIx isoform is expressed in fibers known as IIX for which we have difficulty to distinguish them in cattle from type IIB fibers using conventional histochemical techniques only.

Metabolic characteristics of myosin fibers

The skeletal muscle has as a main function the transformation of chemicals into mechanical energy allowing the production of force for contraction and for maintaining posture. Furthermore, skeletal muscles contribute to basal energy metabolism, thermoregulation and storage of substrates like carbohydrates and amino acids.[3] All these muscle actions use ATP which is produced through three known basic energy pathways in the core of the muscle fiber.[3, 28]

The two main source of energy are carbohydrates by the transformation of glucose and breakdown of glycogen and from lipids that group triglycerides, free fatty acids, ketone bodies volatile fatty and acids. These molecules constitute also within the muscles the reserves that play important role *ante-* and *post-mortem*.[3] The use of these molecules contribute as source for the production of energy for muscle contraction under two complimentary and interconnected pathways: the glycolytic without any molecule of oxygen

and the oxidative pathway that requires the presence of oxygen. After birth and using use at the same time both the metabolic and contractile properties (typing) allow to differentiate 3 fibers types in bovine: the slow oxidative (I) fibers, the fast glycolytic (IIA) fibers and the fast glycolytic (IIX) fibers.[29] These fibers are considered as pure fibers. Other fibers called "hybrid fibers" contain simultaneously several MyHCs. Worthy to note that these hybrid fibers are common in the fetus during the acquisition of contractile properties of fibers.[30] In mammals, they arise from transitions in the expression of MyHCs as fellow: I → I/IIa → IIa → IIa/IIx → IIx → IIx/IIb → IIb. Accoroding to several earlier studies, these reversible transitions occur as a function of age, but also affected by specific factors, namely the activity and exercise of the animals at the farm considered as a rearing practice as well as further rearing factors that include the diet and use of some thyroid hormones.[2, 30-32]

Mean cross-sectional area of myosin fibers

The mean cross sectional area (CSA) of the fibers in cattle and in most muscles is categorized as follows: IIB/IIX>IIA>I (**Table 1**). More particularly, the mean CSA of oxidative fibers is small compared to the glycolytic one allowing an efficient diffusion of O_2.[33] However, exceptions are encountered in particular in some muscles such as *Rectus abdominis* for which the classification is not occurring by the same manner and is likely inverted, the MyHC-I (type I fibers) have greatest CSA.[34] The mean CSA of fibers depends on muscle type. For example, several studies showed low CSA of *Longissimus thoracis.*[7] In an earlier study that used different muscles, the smallest CSA of all fiber types was detected in the *Psoas major* while the greatest was found for the type I in the *Semitendinosus* and for the types IIA and IIX in the *Biceps femoris.*[35]

Techniques allowing the classification of muscle fibers

Muscle fibers are classified using the contraction rate and the metabolic properties characterizing the energy source used by muscle during contraction. These classifications can be performed using very thin (ultra) sections of muscle fibers from a tissue sample frozen in liquid nitrogen and stored at -80°C[29, 36, 37] or using an ELISA test [38] or by adapted electrophoresis using muscle extracts [39-41].

Histochemical techniques

The first histochemical classification as reported by Guth and Samaha [42] used the differential sensitivity of the ATPase activity of MyHC isoforms to acidic or alkaline pH (**Figure 1**). This technique allows distinguishing the fast twitch acid-fast type II fibers from the slow twitch acid-sensitive type I fibers. Indeed, at low pH conditions (acidic), the ATPase activity of fast fibers is inhibited, but not that of slow fibers. In these conditions and as can be seen in **Figure 1a**, the slow fibers are stained with black color, and the fast fibers are in white. Brooke and Kaiser [43] using different pH pre-incubation conditions succeeded to improve this classification by identifying two subclasses of fast fibers (**Figure 1b,c**). A parallel classification as shown in **Figure 1d** was based on joint revelation on serial sections of the activity of ATPase and of enzymes of oxidative metabolism such as succinate dehydrogenase (SDH) [44, 45]. By following this method, slow oxidative (SO) fibers, which are black for ATPase at acidic pH and blue for the SHD activity because of a high oxidative metabolism due to exclusively MyHC I isoform (**Table 1**) can be distinguished. Among the fast fibers, two sub-populations can be distinguished that are the blue fibers under SDH known as fast oxidative fibers FOG (or αR) and contain the isoform of MyHC IIa. The other type of fast fiber (white ATPase at acidic pH) is not colored blue by SDH as this type has a very low oxidative metabolism. These fibers were then called fast glycolytic FG (or αW) containing mainly MyHC IIx. These fibers are known to use carbohydrates to produce energy without oxygen need. In cattle and for a long period of time, IIX fibers were classified as IIB, according to the classification of Brooke and Kaiser [43], because the conventional histochemical techniques were not able to accurately distinguish the two types of fibers.

Immunohistochemical techniques

Immunohistochemistry techniques using antibodies targeting specific MyHCs have been proposed and developed during the last dacade.[36, 37, 41, 46] Therefore, four fiber types (I, IIA, IIX and IIB) can be classified from the detection of MyHC I, IIa, IIx and IIb isoforms (**Figure 2**). This technique allows an efficient differentiation among the pure muscle fibers that contain a single MyHC isoform from hybrid ones containing multiple isoforms. To our knowledge, there exists no other method allowing distinguishing these myosin hybrid fibers. In cattle and according to Picard et al. [29], this technique allows the identification of IIC fibers containing MyHC I and IIa isoforms, and IIAX fibers containing the IIa and IIx MyHC isoforms (**Figure 2**). The identification of these hybrid fibers is of interest to accurately track and analyze the plasticity of muscle fibers.

The image analysis can be performed using different software tools such as Visilog [36] or MyoVision [47] allowing a fast and automatic classification of the fibers after the detection of fiber types based on different antibodies raised against various MyHCs. For example, Visilog a tool efficiently applied by our group allows an objective and fast fiber typing as well as morphometric characterization of cattle muscle fibers. This tool allows saving time and gain reliability for research laboratories due to its combination with the recent advances in fluorescence microscopy, immunochemistry and image processing algorithms. Furthermore, Visilog permits also to automatically determine the mean cross sectional area per type and fiber density (number per mm^2) for an approximate of 300 fibers per serial image. It is further used for fiber typing in other livestock species and in laboratory animals such as rodent [48].

Electrophoretic separation of MyHCs

As stated above, the contractile properties of a muscle could be further analyzed through electrophoretic separation (SDS-PAGE) of the different MyHC isoforms according to their molecular weights. However, these four different isoforms of MyHCs have close amino acid composition and consequently molecular weights (223.900, 224.243, 223.875, and 224.026 kDa for bovine MyHC I, IIa, IIx, IIb respectively), hence there is a difficulty to separate them with a high reproducibility [11]. To avoid this, an accurate protocol was firstly proposed by Picard et al. [40] and another one recently by Scheffler et al. [41] to separate the different bovine MyHC isoforms in mini polyacrylamide gels (**Figure 3**). This technique solved the problems associated to the use of polyacrylamide gradient gels [39] and allows a good separation with a high reproducibility. These conditions were applied successfully on sheep [49-51] and camel MyHCs.[52, 53] The electrophoretic separation allowed identifying a fourth MyHC isoform in some bovine (**Figure 3a**) and identified by Picard and Cassar-Malek [54] as MyHC IIb. From the large literature, it seems that this isoform was considered not to be expressed in the trunk and limb muscles of bovine. The earlier work by Maccatrozzo, et al. [55] showed that the MyHC-IIb coded by the gene *MYH4* was present in the cattle genome on Ch.19, but was not expressed as shown by Chikuni, et al. [56] who described its expression restricted to certain specialized eye muscles. Although the transcript was present in all animals studied, Picard and Cassar-Malek [54] found the MyHC IIb isoform only in some cattle. This suggests the existence of a post-transcriptional regulation in the expression of MyHC-IIb. By the means of an adapted SDS-PAGE electrophoresis protocol[40], MyHC IIb isoform was found with various frequencies among some French beef breeds: Blond d'Aquitaine (25%), Limousin (4%) and

Charolais (6%).[57] More recently, Moran, et al. [58] and Gagaoua and co-workers from two different studies[59, 60] found that the MyHC IIb isoform is present in very few animals only within very different groups of rearing practices and production systems. Using a proteomics based-approach following in-gel digestion with trypsin by LC-MS/MS mass spectrometry, Kim [11] identified peptides specific of MyHC-I, IIa, and IIx, but they did not identify a unique peptide of MyHC IIb in *Longissimus thoracis* muscle of Korean native steers. It is worthy to note that the protocol of Picard et al.[40] allowing a good separation of adult MyHCs but not of fetal and developmental MyHC isoforms (**Figure 3b,c**) compared to earlier protocols [39, 61].

Which is the best classification technique?

From the three examples above, we can see that the selection of the accurate classification method is very important as some authors demonstrated that muscle fibers classification is not strictly the same within each technique. To summarize this and for example, the fibers classified as fast oxido-glycolytic (FOG) following the technique of Peter et al.[45] were considered as fibers containing MyHC IIa isoform characterized with a metabolism to be likely oxido-glycolytic.[5] However, worthy to note that in in some muscles especially the *Longissimus thoracis* considered as reference in most meat science studies, fibers expressing the MyHC IIa isoform and based on an immunohistochemical analysis are classified into two subpopulations according to their metabolic properties: IIA non-oxidative fibers and IIA oxidative.[29] These fibers are categorized as fast glycolytic (FG) fibers based on the method of Peter and co-workers[45], therefore considered as fibers with a glycolytic metabolism due to the exclusive expression of MyHC IIx isoform. In contrast to this, in *Semitendinosus* muscles, all IIA fibers that correspond strictly to FOG fibers have an oxidative metabolism.[29] On other muscles, we can note that the activity of succinate dehydrogenase (SDH) of fast fibers is very low in *Rectus abdominis*, hence making it difficult to distinguish between FOG and FG muscle fibers. So, according to Oury, et al. [62] the classification of Peter et al.[45] cannot be used in the case of *Rectus abdominis* muscle. Therefore, to efficiently classify muscle fibers and characterize the muscles for their contractile and metabolic properties, it is worthy to consider a stepwise approach by first reveal the contractile type using antibodies raised against the MyHC of interest but characterizing the four described above (I, IIA, IIX and IIB) by immunohistochemistry and second evaluate in serial sections prepared following the protocol given earlier by measuring the activity of SDH.[29] In the context of experiments with large number of animals, this

proposed approach based on immunohistochemistry cannot be applied, as it requires lot of time. To avoid this and be able to characterize the contractile and metabolic properties of bovine muscle(s) in another efficient way, the alternative would be the use of protein extracts (homogenates) from fresh or frozen samples taken preferably early *post-mortem* (30 min to 24h) using an adapted protocol of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or other immunological techniques such as ELISA [38], Dot-Blot [60, 63, 64] or Reverse Phase Protein Array (RPPA).[23, 24, 65, 66]. Further, we recommend the measurement of the activities of some enzymes representative of the oxidative and/or glycolytic metabolic pathways [59, 67, 68]. The most commonly tested enzymes from the glycolytic pathway[59] we used are phosphofructokinase (PFK; 2.7.1.11) that catalyzes the rate-limiting step for glycolysis and lactate dehydrogenase (LDH; EC 1.1.1.27) that catalyzes the redox-coupled interconversion of pyruvate and lactate in the cytosol. From the oxidative metabolism pathways, more enzymes can be tested due to the different pathways[59] that are involved such as the hydrolysis of circulating triglycerides, the lipoprotein lipase, the enzymes involved in the β-oxidation of fatty acids known as also as Lynen cycle (a very complex pathway requiring the activation of acyl-CoA synthetase to form fatty acyl-CoA), the enzymes of Krebs cycle (Tricarboxylic Acid (TCA) cycle) related to acetyl-CoA synthesis from fatty acids or glucose such as isocitrate dehydrogenase (ICDH; EC 1.1.1.42), an enzyme that catalyzes the oxidative decarboxylation of isocitrate and producing α-ketoglutarate and CO_2, and also citrate synthase (CS; EC 4.1.3.7) which is the enzyme catalyzing the first reaction of the TCA cycle known described in the large literature as a condensing enzyme. Further enzymes from the respiratory chain and involved in the energy production can be tested such as cytochrome c oxidase (COX; EC 1.9.3.1)[59] that is the membrane-bound terminal enzyme in the electron transfer chain..

Inter- and intra-muscle variability

Among muscles (limb, trunk, and head) large differences were described in the proportions of the different muscle fiber types.[12] The composition in muscle fibers depends on muscle function and anatomical location [8, 31, 69-72]. It has been reported by Rosser and colleagues[73] that the muscles that are deep and involved in maintaining posture are likely to be more oxidative with great amounts of type I fibers than more superficial muscles involved in rapid movements. In cattle, type I fibers occupies a volume 10% higher in the muscles of the front than in the rear, however, the volume that IIX fibers occupies is almost similar with

proportions of 37% and 38%, respectively. An earlier work by Kirchofer, et al. [74] described the composition in fibers of 38 muscles of the beef round and chuck. Thanks to the great developments in the histochemistry techniques by using SDH and ATPase, several authors evaluated efficiently the percentages and cross sectional areas (CSA) of α-red, β-red, and α-white muscle fibers. The chuck muscles were mostly categorized as red (10 of 26) and contain >40% β-red fibers, intermediate (9 of 26), and white (7 of 26), this later with more than 40% α-white fibers. Among 12 round muscles, 9 including *Biceps femoris*, *Semitendinosus*, *Adductor*, *Rectus femoris*, and *Semimembranosus*, were ranked as white. The three other muscles that are *Vastus lateralis*, *V. medialis*, and *Sartorious* were identified and grouped as intermediate. In line to the above, a very recent study by our grouped using the adequate SDS-PAGE[40] cited above and comparing four divergent muscles that are *Rectus abdominis* (RA), *Semimembranosus* (SM), *Longissimus thoracis* (LT), and *Semitendinosus* (ST) showed that among them RA muscle had the highest proportions of type I fibers, followed by LT then by ST and SM that were both equivalent.[8] For type IIA fibers, LT muscle had higher proportions in comparison to SM and RA that were equivalent and with higher proportions than ST muscle with the lowest percentages. Concerning the sum of IIX+B fibers type, the *Semitendinosus* and *Semimembranosus* muscles had the greatest proportions compared to *Longissimus thoracis* and *Rectus abdominis* that were both found to be equivalent.[8]

Intra-muscle variability in the distribution of the different fiber types could be further observed. Generally, the proportion of the IIX fibers is much higher in the posterior and superficial muscles whereas the proportions of the type I fibers is greater in the medial and anterior muscles.[72] Accordingly, a proximal-distal gradient in terms of the metabolic and contractile properties was reported in an earlier study by Brandstetter and co-workers[75] in young cattle along the length of the *Semitendinosus* muscle. The fast glycolytic type decreased and inversely the slow oxidative type increased from the proximal to distal extremity, and this gradient was found to disappear in 16 months old young bulls [75]. Also, Hunt and Hedrick [76] who investigated the fibers in 5 bovine muscles from six steer beef carcasses of the USDA choice and taken at a commercial slaughterhouse with typical A maturity of no physical evidence of double muscling and with uniform distribution of the marbling content and normal color, found in ST and SM muscles that the inner part of the SM was richer in glycolytic fibers than the outer part and the inner part of the ST was richer in oxidative fibers than the outer. Moreover, a very recent study by Van Bibber-Krueger and

co-workers on young black-hided cattle reported around 11.6% less type I fibers and 14% more type IIX fibers in the proximal location of the ST muscle when compared to the middle and distal location, designating this location to be more glycolytic than the others[77]. Further, the authors highlighted that the middle location had 5.4% less type IIA fibers and 8.7% more type IIX fibers than the distal area, describing this later to be also glycolytic. Using an histochemical technique, as described in the methods above, the authors confirmed their results by assessing the SDH staining intensity[77].

Finally, it is worthy to consider that the proportions of the three or four main fibers we characterize within muscles are further highly variable regarding to species. As example, the slow oxidative muscle *Masseter* of adult cattle contains exclusively 100% type I fibers.[78] This composition that we consider coherent can be explained by the physical role this muscle plays rumination and mastication in ruminants. However, for the animals that are characterized with faster mastication profile especially in rodents such as mice, rat, and rabbit, the *Masseter* muscle is composed of both the slow oxidative I and oxido-glycolytic IIA fibers. In other species such as guinea pigs (*Cavia porcellus*), this muscle contains only IIA fibers. On another hand, an unusual fiber type that contains a super-fast MyHC isoform that had a gene name *MYH13* was observed in carnivores.[27] At the end of gestation, this muscle is composed of approximately 80% fast and 20% slow fibers. In suckled animals, the percentages of slow fibers was described to increase with age from approximately 53% at the age 170 days to 100% just after weaning in different gender including steers, bulls and cows.[78] These data illustrate that the slow-twitch properties of this muscle are acquired due to an adaptive response to rumination, hence, demonstrating that the properties of the fibers depend on the rate and extent of the contractility of the muscle. The *Diaphragma* muscle is the other example that we can cite here. This latter was described to contain mainly IIX fibers and considered as fast muscle in mice and rats, but as a slow muscle in large mammals including cattle and in which it contains both type I and IIA fibers.[15]

Breed variability

For cattle, it was demonstrated that the muscle properties are breed-dependent, as observed in other species. A comprehensive study on 30 bulls from 15 breeds in Europe with similar rearing practices and slaughtering conditions, illustrated the various properties of *Longissimus thoracis* muscle.[67] Among the 15 breeds, especially those of Spanish origin (Casina, Avilena) known as hardy beef breeds were found to have more oxidative muscles;

the fattiest breeds of British origin (Hereford and Aberdeen Angus) or Jersey known as a small-size dairy breed had more oxidative activity and slow I and IIA fibers with high glycolytic activity and a low proportion of IIX fibers. On the contrary, the lean breeds such as Limousin and Charolais of French origin and Piedmontese, an Italian-born breed had high proportions of fast glycolytic fibers IIX.[67] The comparison of even more extreme breeds such as double-muscled Belgian Blue comparatively to Angus, confirmed these trends in the distribution of the muscle fibers in *Longissimus thoracis* muscle with higher percentages of fast glycolytic fibers in Belgian Blue and oxidative metabolism with lower IIX fibers in Angus. These properties of oxidative metabolism with lower or no IIX fibers were further observed in the two breeds of the official French quality label "Taureau de Camargue" that are Brave and Di Biou.[79] Overall, the data presented above clearly highlighted that the objective of selection of cattle for meat production is likely to induce a higher percentages of the fibers IIX (fast glycolytic) related with less oxidative metabolism and glycolysis. Accordingly, a comparative study[71] LT muscle of Aberdeen Angus and Limousin breeds showed strong differences in the proportions of all the isoforms of MyHC-I, -IIa and -IIx/b. The percentages of the fast oxido-glycolytic MyHC-IIx/b was found to be the highest in the Limousin breed and the other fibers were the highest in Aberdeen Angus. In support of what we presented above, a meta-analysis conducted on large number of cattle metadata including different breeds (Aubrac, Salers, Charolais, Montbéliard, Holstein and Limousin) highlighted the trend in the variations existing in muscle fiber type proportions.[7, 69] The key home message from the two meta-analyses is that French beef breeds grouping Limousin, Charolais, Salers and Aubrac had a metabolism that is likely glycolytic and associated with a higher percentages of fast glycolytic fibers compared to dairy breeds that are Holstein and Montbéliard. Mixt breeds such as Rouge des Prés have intermediate properties.[80, 81] The same conclusion was very recently reported under industrial conditions whatever the gender or age of the animals.[8]

Myogenesis and muscle development

Skeletal muscle development occurs in three well defined stages that are the embryonic, the fetal and the adult.[16] During the prenatal stages, the development of skeletal muscle is comprised of the formation of three main components that are i) muscle fibers known as myogenesis; intramuscular adipocytes known as adipogenesis; and the formation of iii) fibroblasts known as fibrogenesis.[16, 82] These described cells that have pivotal role on the meat

properties outcomes has a common origin that is the pool of progenitor cells that develop during the embryonic stage. At the early embryogenesis phase, cells known as multipotent mesenchymal stem diverge into either adipogenic fibrogenic or myogenic lineages. On another hand, the myogenic progenitor cells in turn develop into satellite cells and muscle fibers whereas the adipogenic fibrogenic lineage cells develop into the stromal-vascular fraction of skeletal muscle that group both the fibroblasts and the adipocytes. At the whole muscle level, it is the number and the size of individual muscle fibers that determine the size of a muscle. For further details, we recommend several references on cattle myogenesis.[15, 17, 83, 84] In the following sub-sections, we describe the properties of the different muscle fibers during this important phase of myogenesis and muscle development along the life of an animal.

Fetal life

Fetal muscle development involves two phenomena, the increase in muscle cell numbers that is hyperplasia, and of their size or diameter (cross sectional area and length) known as hypertrophy.[85] In cattle, a minimum of two generations of fibers were well described to be involved in the myogenesis stage (**Figure 4**). The primary generation of cells from the embryonic myoblast fusion, was observed from 30 days post-conception (dpc) and was fully differentiated at the end of the second trimester that is about 180 days of gestation. At this period, the slow twitch fibers become Type I fibers in most muscles of the body, except in adult fast muscles such as the *Cutaneus trunci*, where they are mainly converted into fast fibers.[15, 83]

The second generation of cells during the fusion of the fetal myoblast was detectable from the end of the first quarter, and was in great part at the origin of IIX fibers. The conversion of the secondary fibers to slow or fast fibers in a muscle-dependent manner occurs just after birth.[15] Some studies suggested that third generation of cells is present as revealed using an immunohistochemistry approach based on specific antibodies against MyHCs.

The study of certain markers of cell differentiation revealed that the metabolic and contractile maturation of muscle fibers occurs mainly during the last trimester of gestation.[15] During this period, the expression of developmental MyHCs isoforms was decreasing. The isoforms (including embryonic, α-cardiac and fetal) revealed by immunohistochemistry (**Figure 4**) or by an adapted SDS-PAGE electrophoresis (**Figure 3b,c**) are gradually changed

411 by the adult isoforms of fast myosin heavy chains. The slow fiber isoform of myosin heavy
412 chain (MyHC) is expressed very early, and shown to be detectable around 30 dpc in
413 myotubes, and then it is the only muscle fiber that is expressed from the first generation from
414 180 dpc. During the last trimester of gestation, this isoform of MyHC is also detected in the
415 fibers of the 2nd and 3rd generation, which are in adult muscle at the origin of type I fibers.
416 According to Picard and co-workers, three weeks after the birth, the muscles of cattle contain
417 only type I, IIa and IIx adult MyHC isoforms[83], and they no longer contain fetal or
418 developmental MyHCs. In a particular manner, the contractile properties of bovine muscle
419 fibers get their maturation early at birth, in a similar manner than human[86] and sheep[87] but
420 unlike of rodents[88].

421 Earlier studies reported that during the last trimester of gestation, an increase in the
422 activities of enzymes from the glycolytic and oxidative pathways occurs from 180 or 210
423 dpc.[15, 83] Therefore, from these stages of gestation, muscles can be easily distinguished thanks
424 to their metabolic peculiarities. Compared to other species such as rats, chickens, pigs or
425 rabbits this was possible only during the month following birth or hatching.[15] In cattle, the
426 metabolism of all type I fibers from the first generation is oxidative from 210 dpc. For the IIA
427 fibers, an increase in the oxidative metabolism is observable during the last trimester of
428 gestation, and the whole of the fibers were found to be characterized from birth with oxidative
429 metabolism.[83] During the last trimester of gestation and in parallel with changes in the
430 expression of the different myosin isoforms, in increase in the activities of the glycolytic
431 enzymes was also reported such as the conversion of cardiac to skeletal lactate dehydrogenase
432 (LDH) isoforms.[89, 90] To deeper our understanding all these above important changes that
433 occur during the last trimester of gestation, a comprehensive proteomic experiment was
434 performed on *Semitendinosus* muscle at key stages of fetal life that were 60, 110, 180, 210
435 and 260 dpc.[89, 90] This serial time study highlighted hundred proteins changing during
436 gestation which confirmed a proliferating and fusion activity of muscle cells between 60 and
437 110 dpc, especially with a high production of proteins involved in mRNA processing and
438 developmental processes including splicing, such as Heterogeneous nuclear ribonucleoprotein
439 H3 (HNRH3) and Apolipoprotein B mRNA editing enzyme. This earlier proteomic study
440 further confirmed the key stage of 180 dpc with a high abundance in the production if
441 particular proteins such as WARS (Tryptophan--tRNA ligase), PARK7 (Protein/nucleic acid
442 deglycase DJ-1) and CLIC4 (Chloride intracellular channel protein 4). These proteins were
443 proposed by the authors as putative biomarkers of the total number of fibers.[89, 90] Stages from

201 to 260 dpc were characterized as differentiation and maturation stages in relation to the increase in the abundance of proteins related to the energy metabolism pathway. It is worthy to note that modifications in isoforms of contractile proteins were further observed.

Postnatal growth

Post-natal muscle growth occurs through muscle fibers hypertrophy (**Figure 5**) as described in other vertebrate species [91, 92]. For example, the postnatal growth of *Semitendinosus* muscle from birth to 24 months of age was found to be characterized by an increase that is nearly 10-fold of muscle fiber cross sectional area (CSA). This muscle hypertrophy is originated from the fusion of muscle satellite cells with existing fibers. Satellite cells, originating from the embryonic myotome are situated between the basal lamina and the sarcolemma, playing an important role in the regeneration of muscle fibers and their growth. In cattle fetuses, these cells were found to be detectable at 65 dpc[82] and at 85 dpc in sheep [93]. In the muscles where these cells exist, their fusion and proliferation contribute to the continuous increase in the amount of nuclei in muscle fibers of adults.[94] In this context, some studies in the field discovered that in adult muscle the density of satellite cells depends on the myogenic activity during fetal life. A reduction in both the density of satellite cells and the number of muscle fibers was observed and associated to a significant decrease of myogenesis during fetal development, hence inducing permanent reduction of muscle mass [93, 95].

In a comparison between *Biceps femoris* muscle from Angus and Wagyu cattle of 12 months old, a study by Fu, et al. [96] demonstrated that Aberdeen Angus had higher satellite cells density compared to Wagyu, a highly marbling breed. However, Wagyu breed had larger fibers, suggesting a lower number of fibers in this breed comparatively to Angus, thus an attenuated myogenesis during early muscle development occur in the marbled cattle. As intramuscular adipocytes and myofibers had as origin the same pool of mesenchymal progenitor cells, the authors suggested a shift in Wagyu cattle from myogenesis to adipo/fibrogenesis during early embryonic development phase.

The post-natal muscle growth in cattle is related with modifications in the metabolic and contractile properties of muscles in two main phases (**Figure 5a**). From 0 – 12 months corresponding to the first phase with intense muscle growth, the oxidative metabolism decreases and the glycolytic metabolism increases.[83] These modifications are related to changes in muscle fibers mainly with a decrease in the percentages of the fast

oxido-glycolytic IIA fibers and an increase in the percentages of the fast glycolytic IIX fibers. Type I fibers are slightly modified by age. This evolution continues in males until 12 months and then we assist to a gradual reverse. In the second phase after 12 months, a slowdown in the intensity of muscle development and growth is associated with a decrease of the percentages of IIX fibers and an increase of those of I and IIA fibers. Based on a meta-analysis, Schreurs and co-workers [69] were able to perform a modeling of the changes in muscle fibers proportions and cross section area with age and according to factor such as breed (**Figure 5b**). These modifications with age in muscle fibers characteristics interested several groups and evidenced that they can be modulated by numerous factors related to production such as diet, the physiological state of the animals as well as their physical activities.[5, 31]

Regulation of muscle fiber plasticity

As described in the previous sections, the specification muscle fiber type begins prior innervation in the embryo. After birth, a shift of muscle fibers to an overall fast or slow phenotype is induced under a neural influence. This is referred as "muscle fiber plasticity" [97]. Many excellent articles have reviewed the biological processes involved in both specification and plasticity of fibers which will not be detailed in this review.[1, 97-100] Several factors can modulate or influence the proliferation of myogenic precursor cells due their sensitivity endocrine regulation and nutrients; thus, the physiological conditions and maternal nutrition affect abundance of myogenic cells and their proliferation and consequently the subsequent development or formation of muscle fibers.

Fetal programming

Skeletal muscle development is especially vulnerable to nutritional change.[95] In cattle, mid-gestation at the prenatal stage was described to be paramount importance and critical for the development skeletal muscle and for the determinism of the future muscle growth potential.[16, 93]. In fact, maternal nutrient deficiency during this stage induces a significant decrease in the number of muscle fibers. In cattle and sheep and at the late gestation stage, maternal nutrient restriction was found to be with no damageable effect muscle fibers numbers.[16] However, at this stage maternal nutrient restriction could induce a decrease in the muscle fiber size [93], hence impacting the postnatal muscle growth leading by lowering the population density of satellite cells.[101] Several studies showed that during gestation both an

over-nutrition or malnutrition impact offspring growth performance.[17] As the total number of muscle fibers is determined during fetal life, any decrease in muscle fibers content as a consequence of fetal programming, immediately decreases the muscle mass and adversely impacting animal performance. Accordingly, a recent work by Ward, et al. [102] investigated the transcriptome of hind limb fetal muscle in Angus-cross heifers with maternal nutrient restriction during early gestation since 50 days. They found 22 genes differentially expressed between the restricted and control groups. Among them, the authors identified the myogenic genes myoblast determination protein 1 (MYOD1) and myogenin known for their regulation of fiber development and skeletal muscle cell differentiation. From the Wnt signaling pathway, 4 members were found to be up-regulated in restricted fetuses. It is worthy to note that in line to the observation of Kaspric, et al. [103] using bioinformatics and proteomics approaches, the Wnt pathway is very important concerning the promotion of myocytes differentiation. Additional genes were upregulated in restricted fetuses including members of the troponin, myosin and actin proteins. Recently, an analysis of the *Semitendinosus* and *Longissimus thoracis* proteomes of fetuses from heifers subjected to restriction in nutrients and then to re-alimentation from early to mid-gestation reported further insights. Thus, the changes in the abundance of a total of 28 proteins mainly related to protein metabolism or to glucose, in the regulation of cell proliferation or apoptosis was modified by maternal nutrition [104].

Genetic variability in the metabolic and contractile characteristics of muscle fibers

Myostatin, MSTN, also called growth differentiation factor 8 (GDF8) is one of the important regulators of myogenic cell proliferation and is a highly negative regulator of skeletal muscle mass [105]. MSTN regulates both muscle mass and fiber type composition. MSTN is first translated as a secreted protein which is at this stage in an inactive form. The activation occurs after that that through two separate cleavages to remove both the inhibitory domain and signaling peptide. This leads to the production of an active form of MSTN able to bind easily to several regulatory proteins present the blood.[106] MSTN is first expressed in the somites of the myotome and later in developing fully skeletal muscles. Absence of functional MSTN during fetal development induces a significantly increase in the adult muscle mass through both hyperplasia and hypertrophy of the skeletal muscle myofibers. In adult animals, a differential expression of MSTN according to the type of muscle has been described, fast muscles and especially those composed of type II fibers contain greater levels of MSTN than

538 slow muscles.[107] MSTN is further involved in the regulation of the function of muscle satellite
539 cells, by playing a role in their inhibition in several animal species.[106]

540 In cattle, mutation in MSTN results in "double muscling" phenotype.[108, 109] For example,
541 in double muscled Belgian Blue breed a high hyperplasia of muscle fibers during fetal life
542 originates around twice the total number of fibers compared to other breeds.[108, 109] The type I
543 fibers number was not affected, therefore suggesting that the additional fibers found in the
544 postnatal double muscled Belgian Blue were type IIX and IIA fibers. Further studies on fetus
545 confirmed a higher proliferation of the fibers related to the second generation, hence leading
546 to a higher percentage of the fast glycolytic fibers.[110] In line with the negative control role that
547 MSTN plays on the proliferation of fast-twitch glycolytic muscle fibers, a proteomics analysis
548 on *Semitendinosus* muscle from adult double muscled Belgian Blue showed a higher
549 abundance of proteins of the fast glycolytic type in homozygote cattle comparatively to their
550 controls, and the heterozygotes being intermediate.[111] The authors identified some proteins as
551 candidate biomarkers of muscle hypertrophy, among them the Myosin-binding protein H
552 (MYBPH) that is a protein of approximately 55 kDa and encoded by a single gene (*MyBPH*)
553 expressed in both cardiac and fast skeletal muscle cells.[112] Moreover, the study showed a
554 modification of alternative splicing of the fast skeletal muscle fTNNT (Troponin T).[111] The
555 expression of fTNNT exon 16 structure was increased in double muscled Belgian Blue
556 muscle whereas fTNNT exon 17 was unchanged. These results suggest an important role of
557 the exon 16 of the fTnT in the physiological adaptation of fast muscle characteristic (**Figure**
558 **6**). Using a transcriptomic approach, another study compared the transcriptome of fetuses
559 from two French beef breeds: Blond d'Aquitaine breed with muscle hypertrophy in adult and
560 muscle properties similar to those of double muscled cattle[113] and Charolais breed used as a
561 control. The results showed in Blond d'Aquitaine a transition to a fast glycolytic muscle
562 phenotype detectable beyond 210 dpc through down regulation of various slow twitch subunit
563 proteins such as TNNC1, MYH7 (Myosin-7: slow MyHC-I), TPM3 (Tropomyosin alpha-3
564 chain) and cysteine and glycine-rich protein 3 (CSRP3) known as cardiac LIM protein (CLP)
565 or muscle LIM protein (MLP).

566 As observed for double muscled cattle, it was shown that adult in Charolais selected
567 based on their muscle growth potential into high (H) *versus* low (L)[114] to have greater
568 percentages of the fast glycolytic fibers. Analysis of H and L fetuses along gestation
569 highlighted a higher hyperplasia in H muscles. This was further confirmed by *in vitro* studies

showing a higher proliferation of myoblasts from high potential growth muscle[115] as observed for double muscled comparatively to non-double muscled myoblasts.[83] It is worthy to note that a higher fast glycolytic phenotype in H muscles was observed from the last trimester of fetal life. Nevertheless, it was further shown that a delay in the physiological maturity, consequently inducing a delay in the plasticity of muscle fibers after birth and observed in H animals compared to L cattle. It is important to mention that the effects of selection on muscle growth potential are observable only at some ages of the life of the animal due to different kinetics of muscle growth that can be observed in H and L cattle.[30] Similarly to the observations descried for double muscled animals, differences in the expression of fTNNT were found in H cattle comparatively to L cattle. These data highlight that there exist a strong association in the increase of muscle and the fine regulation in the expression of fTNNT isoforms. It appears that the greater the muscle mass of the animals is, the higher is the value of the ratio fTNNT exon 16 / fTNNT exon 17. According to these results the proteomic work by Bouley et al.[111] suggested this ratio as a good indicator of muscle mass (**Figure 6**).

Hormonal factors influencing muscle fibers properties

The effect of androgens was extensively described in the large literature, indicating muscle hypertrophy originated by a higher CSA of all fibers in bulls comparatively to steers.[116, 117] These properties are associated to the slowdown with age of the conversion of the fast oxido-glycolytic IIA fibers into the fast glycolytic IIX fibers. Muscles of steers comparatively to bulls at the same age have more amounts of fast glycolytic fibers IIX, and higher glycolytic activity of the enzymes characterizing the glycolytic pathway.[58, 118-120] On another hand, steers were reported by some studies to have lower percentages of type IIX fibers and greater percentages of type IIA compared to heifers whatever the age.[7, 59, 121] However, the differences are more or less pronounced depending on the muscle.[8] In this context, the studies that investigated the effect of the age at castration at 2 months or 4 months revealed that the consequences on muscle fibers properties were observable only after puberty.[118] These authors showed that testosterone production started at almost 2 months, however, no difference in muscle fiber composition was observed nor at 4 neither at 8 months. At 12 and 16 months, the muscles of steers contained more percentages of IIX fibers and lower proportions of type I fibers compared to young bulls. These differences further confirmed in steers to be related with a lower oxidative activity essayed by isocitrate dehydrogenase (ICDH) and a higher glycolytic activity as assessed by lactate dehydrogenase

(LDH). Moreover, the average cross sectional area (CSA) of the fibers was lower in steers that is explained by the hypertrophic role or impact induced by testosterone in bulls.[118] Muscle hypertrophy can be further induced using β-agonists (exogenous anabolics) such as zilpaterol, cimaterol and clenbuterol. In animals treated with these chemical agents, the average CSA of fibers in their muscles increased and contain lower percentages of the slow oxidative type I fibers and faster glycolytic type IIX fibers compared to their controls.[122-124] It is worthy to note that this effect included an increase in the CSA of the type I fiber.

Nutritional effects

In addition to the previous sections, the efficiency in muscle growth of cattle can also be manipulated through the diet applied during postnatal period. In fact, among the important factors that were extensively studied as influencing factors in cattle production of the muscle fibers properties we cite the composition of the diet and its energy level.[31] Intensive research in this context exist in the large literature.[121, 125-127] For example, the dietary restriction before weaning induces modifications in muscle fibers characteristics, which are still visible 5 months after weaning (*i.e.* at the age of 9 months) but are not yet observable at 18 months which is the age at slaughter.[118] Moreover, the CSA of fibers was found smaller 5 months after the end of the restriction and the percentages of type IIX and I fibers were higher in the muscles of restricted animals compared to their controls.[83] These characteristics are the consequence of the phenomenon of the compensatory growth described by Hornick, et al. [128]. This is current in extensive production systems where animals alternate periods of adequate feed supply from periods of insufficient nutrition.[129] Most of the studies that we cited in this section and all dealing with the impact of energy restriction after weaning followed by compensatory growth, reported a decrease in the cross sectional area of the fibers accompanied by an increase in the percentages of the oxidative metabolism fibers (type I and IIA). In contrast and as expected the activity of enzymes of the glycolytic pathway and the proportions of IIX fibers decreased. An earlier study by Yambayamba and Price [130] in heifers suggested that a decrease in the energy intake of the animal leads to more oxidative fibers than glycolytic fibers in the *Longissimus thoracis* muscle.

The opposite trend was observed during the period of compensatory growth, where an increase in the percentages of the fast glycolytic IIX fibers accompanied with a more glycolytic metabolism is dominating compared to all what is oxidative that strongly decrease.[118, 127] Similarly, the CSA of the fibers were affected at the end of the compensatory

growth and remains lower compared to the controls. Several factors such as the genotype, the gender or sex, the type of muscle including the metabolic changes, the maturity level and fat amount of the animal thus of its muscle at the time of nutrient deprivation, the age of the animals as described above impact compensatory growth.[125] The intensity and duration of the restriction and compensation play both a great role.[131] Variations in growth rate during the finishing period can also induce changes in muscle properties.[129] For example, Vestergaard, et al. [132] found in *Semitendinosus* muscle of animals produced extensively an increase in the IIA fibers percentages. On another hand, the studies that investigated the impact of the nature of the diet reported that grazing cattle have as expected more oxidative muscles than those receiving only corn silage.[68]

In the large literature, most of the factors cited above were investigated including a combination of some of them in one study such as the respective impact of the type of diet (or its nature) with the activity (mobility) of the animals at the farm. Therefore, the oxidative properties of the muscles were then evidenced to be related to the activity of the animals that move rather than to the strict effect of type of feeding regimen, as physical activity induces conversion or a switch of the fibers from white to red.[31, 125] In this context and in line with these statements, an elegant comprehensive review by Dunne and co-workers explained that the changes in color of the muscle fibers is likely to be due to the higher physical activity of the animals at the farm as the muscles of these cattle reared on pasture contain greater amounts of myoglobin in response to the high percentages of the oxidative fibers.[133] More recently, the study by Gagaoua et al. [31] on PDO Maine-Anjou cows identified that on animals reared mostly under grass feeding regimen had higher percentages of oxidative IIA fibers at the expense of IIX fibers (**Figure 7**). Also, studies by Gagaoua et al. [31] and Picard et al. [66] revealed that further proteomic biomarkers can change with muscle fibers as their expression is interrelated. Further, a recent study showed that intramuscular fat content decreased and the percentages of IIA fibers increased in pre-finishing animals with a grazing period compared to those pre-finished with only on concentrates.[58]

Overview of associations between muscle fibers typing and beef quality traits

From the large literature and whatever the species, several meat qualities such as pH decline, tenderness, juiciness, flavor, color, drip loss, water-holding capacity (WHC) and marbling are associated to the different characteristics of the muscle fibers described in the sections above and cited references.[2, 5, 6, 32] In cattle, the associations were mainly described

for pH decline, color and beef tenderness among the other qualities that we briefly summarize in the following sub-sections.

pH decline

The classification of muscle fibers by contractile speed (slow *vs.* fast) and metabolic properties (oxidative *vs.* glycolytic) is the reasons for which MyHC isoforms were directly involved as drivers of the early rate of *post-mortem* metabolism[6] including pH drop.[81, 125] It is well established in red muscles that the rate and extent of pH decline can be higher in meat with more percentages of fast-twitch glycolytic fibers.

Associations of MyHC isoforms with pH decline are partly related to glycogen at slaughter and to the mitochondria contents[134] that differ among the different muscle fibers.[5] For example, in the oxidative fibers IIX and IIB, there is lower glycogen content responsible of the decrease in the rate of glycolysis, and thus slower rate of pH decline due to a slower accumulation of lactic acid.[125] Further, the glycolytic rate influences *post-mortem* changes to myofobrillar proteins such as myosin, actin, troponin, and some metabolic proteins, particularly glycolytic enzymes in the sarcoplasm, and these *post-mortem* protein changes can influence the ultimate meat quality (tenderness, juiciness, flavor or color development/stability). Conversely, oxidative slow twitch fiber types generally have lower glycogen storage (**Table 1**) and tend towards having a high ultimate pH, which is associated with lower lightness of meat, due to both reduced light scattering [135, 136] and higher oxygen consumption in the surface.

Several studies reported correlations between muscle fibers including the muscle type and pH decline. For example an earlier work by Whipple, et al. [137] reported that the muscles that had more oxidative properties had higher pH measured at 3 and 12 h *post-mortem*. In another study amongst LT and RA muscles of cows, both IIA and IIX fibers were correlated positively and negatively with ultimate pH measured at 24 h *post-mortem* in LT and RA muscles, respectively.[81] It is worthy to note that, muscles that contain more proportions of type II fibers are more susceptible to *post-mortem* glycolysis than those that had more percentages of the oxidative type I fibers.[4, 138] Likewise, the muscles with greater percentages of type IIX+B fibers and thus lower type I fibers were described to have higher contents at 45 min *post-mortem* of glycogen and lactate.[139]

Muscles with different fiber type percentages (composition) including individual variability among animals of the same herd, have different patterns of *post-mortem* change during the period the conversion of muscle into meat, and may have further significant impact on the meat quality traits such tenderness and color.[140-142] Overall, from the large literature there is on one hand scarcity in studies that investigated in different cattle type the associations that exist between pH and muscle fibers and on another hand, from those that we reviewed it seems that the relationship between pH values and muscle fiber types is more complex than expected. To better understand the driving factors, we suggest in depth characterization using a multi-OMICs and holistic approach by combining for example genomics, proteomics, metabolomics in an integrative modeling manner to be able reveal the main changes that occur under different conditions and factors described above.

Color

Meat color is critical to fresh beef marketability as it influences consumer purchase decisions and attractiveness at the moment of purchase.[143] The cherry-red as an optimum surface of beef color, is what consumers consider as a guarantee of freshness on a meat product. Overall, the redness of meat depends on its myoglobin that is mainly found in oxidative red fibers[5] (**Table 1**). The myoglobin content and the rate of its oxidation were described to be muscle-specific.[6, 144] So, the red color characterizing meat is mostly associated to the percentages of the oxidative fibers in the muscle[76], but factors other than myoglobin chemistry are also responsible of the variation of color.[136] Among them, the role of muscle proteins have been extensively investigated including the pivotal role of fiber types[71, 145], mitochondria and sarcoplasmic proteins[146, 147] and myofibrillar structure.[71, 148] *Post-mortem* muscle pH decline further had an impact on the changes in muscle fibers, hence inducing changes in the degree of light penetrating of the structural elements of the meat matrix including muscle fibers, therefore impacting both the development and stability of meat color.[147, 149] The correlation of MyHC isoforms with color traits was described in several studies and this may further reflect the role of metabolic enzymes in color development and stability. As extensively developed in the sections above, the red slow-twitch fibers have higher mitochondria density (**Table 1**) including the enzyme systems that allow oxygen consumption and electron transport chain.[4, 144, 150, 151] The study by Jeong et al.[152] who investigated the discoloration trends in three muscles that are the *Semimemebranosus* the *Longissimus dorsi* and *Psoas major* from Hanwoo breed found that higher percentages of the

oxidative type I muscle fibers are linked to quicker discoloration due to increased oxygen consumption rate, while muscles of increased color stability were mostly comprised of glycolytic white fibers. Type I fibers compete with myoglobin for oxygen, thus making it less available and therefore affecting color determination. Further, earlier studies evidenced that the oxygen consumption rate of muscles is related with the amount of mitochondria available in muscle fibers and may be inversely related to color stability.[145] Accordingly, Tang and colleagues showed that higher amounts of mitochondria increased the oxygen consumption rate, hence the formation of DeMb at the expense of MetMb that decreased.[153] Meat color depends further on the glycolytic activity [147, 154], oxygen consumption and reductive enzyme activity in the *post-mortem* muscle.

The proportions of muscle fiber types differ across muscles, breeds and genotypes and this may explain the different associations that we observe in the large literature.[5, 9, 23, 31, 71, 77, 81] In addition to differences in enzymes and associated reducing capacity through enzymatic and non-enzymatic mechanisms and to the mitochondrial oxygen consumption, various muscle fiber types also contain different proportions of pigment other than myoglobin, of glycogen and lipids, which may also influence meat color.[144, 145] It is worthy to note that, the oxidation of lipids and myoglobin are closely associated in the meat matrix with an increase in one resulting inevitably by similar increase or trend for the other, hence affecting meat color.[155, 156] Oxidation was described in the last decades to be directly associated with the oxidation of myoglobin or to the destruction of its reducing systems by free radicals produced during lipid oxidation and associated reactions.[155] Thus, variations in levels of glycogen and in glycolytic and oxidative enzymes would also be expected to be associated with variations in beef color, but through a different mechanism to myoglobin.[147] Muscles that contain reduced percentages of the type I fibers had greater lightness (L^*) values.[9, 71, 157]. These fibers have also more proportions of myosin light chains that are members of the myosin light chain family.[111, 158] Other factors related to the rearing practices of the cattle can be also at the origin of the associations between color and muscle fibers as evidenced by Gagaoua and co-workers[31] (**Figure 7**). In accordance with earlier reports as reviewed by Dunne, et al. [159], animals with higher physical activity at the farm namely those reared under grass, greater amounts of myoglobin characterize their muscles in response to the high oxidative fibers developed as a consequence of the shift in the muscle fibers described previously.[31, 68] Further, protein markers can be also affected as their expression is associated to the proportions of the different muscle fibers and respectively correlated with beef color traits.[147]

Finally, it worthy to mention that studies from the large literature have further reported that the typing muscle fibers would play a role and consequently influence the muscle susceptibility to the formation of dark-cutting meat known also as dark, firm, and dry meat (For review: Ponnampalam, et al. [160]).

Following the sections above, beef color, especially its stability, can be further affected by the location on the muscle, i.e., intra-muscle effect. This can be exemplified by steaks from ST muscle. Accordingly, Van Bibber-Krueger et al.[77] postulated that the distal steaks of ST muscle had smaller muscle fiber CSA, especially smaller type I CSA, and indicating close relationships within mitochondria, hence increasing the increased oxygen consumption and impacting the color and its stability.

Tenderness

In beef cattle production, animal growth and meat quality are the two main factors of paramount economic importance. As extensively presented above, an increase in the amount of muscle mass can be reached by increasing the size of the fibers and/or via the shift (plasticity) of the slow-twitch into fast-twitch fibers[116], consequently affecting meat quality traits including tenderness. This was for example evidenced in *Longissimus* muscle by studies dealing with the effects of growth path and potential on beef tenderness from different animal types.[161] Others investigated also, the mechanisms by which the impact of altered growth rates would play a role, namely on the calcium-dependent proteolytic system (calpains) and links with the tenderness of beef steaks.[162]

Beef tenderness was extensively investigated due to its importance for both consumer satisfaction and repurchasing decisions.[3, 163, 164] Among the biochemical properties of the muscles such as intramuscular fat, connective tissue components including collagens, muscle fiber properties were the more extensively components that were investigated for their relationships with tenderness and texture traits of beef.[4, 7, 13, 59, 165-167] The associations are complex and vary according to numerous factors such as muscle, age at slaughter, sex or gender, breed and animal type, cooking temperature and evaluation method of tenderness.[5, 6, 9, 26, 31, 58, 59, 62, 68, 165] For example, in a recent study on three your bull breeds (Blond d'Aquitaine, Aberdeen Angus and Limousin), and irrespective of the end-point cooking temperature (55°C usual in France and 74°C usual in British countries) and origin of panelists, beef tenderness was found to be correlated with the fiber types I, IIA and IIX, but

with divergent directions.[168] The natural heterogeneity in muscle fiber type among muscles was further described to affect the final outcome of beef tenderness.[25, 169] Indeed, muscles that are composed of fast fibers type II (higher percentages) are more susceptible to early *post-mortem* proteolytic degradation than those composed by slow type I fibers.[170] As described above, IIX fibers that have greater glycogen contents[2], which together with their specific enzymatic characteristics influence the rate and extent of pH drop, and consequently the final tenderness.[134]

Further, a recent study on Nellore cattle by Chardulo and co-workers[167] who combined different methods to characterize the meat matrix by using physical and chemical analyses as well as molecular biology tests, indicated *MYH2* and *MYH1* genes expression was lower in heavy compared to light animals and MyHC-I was more abundant in tough *versus* tender meat. The authors further reported elevated percentages of MyHC-IIa in the tender meat group (negative correlation with Warner-Bratzler shear force) and its use a biomarker of meat quality in Nellore cattle.[167]

In young bulls of three continental breed that targeted ribeye steaks (LT muscle), the proportions of IIV fibers quantified by DOT-BLOT were proposed as a robust biomarker of tenderness irrespective of the sensory panel, the evaluation method of tenderness or instrumental based on Warner-Bratzler shear force.[64] Using proteomics and as recently reviewed by Picard and Gagaoua [26], several entities of the myosin fibers were identified as potential biomarkers of tenderness. Among those studies and proteins we cite MyHC-I [165, 171-173], MyHC-IIx [165, 171, 174-176], Myosin regulatory light chain 2 (MYL2) [173, 176], MYBPH [175, 177, 178], MYL1[177, 179-182], myosin regulatory light polypeptide 9 [183] and myosin light chain 3 [184].

Conclusion

In summary of this comprehensive review, it seemed that most of the data available in the large literature evidenced the importance of the contractile and metabolic properties of bovine muscle fibers. They further showed that these muscle properties are continuously modified throughout the life of the animals and also on the whole continuum from the farm to meat as a consequence of myriad factors likely the age and the age at slaughter, the sex or gender, breed, rearing practices and production systems. These factors that have different levels of impact on the muscle growth and development and on the fiber properties (*i.e.,* oxidative *vs.* glycolytic) play paramount consequences on several beef qualities. Overall, this review shows

that it is very important to control muscle growth/development and its plasticity for cattle production in order to manage efficiently the desired carcass characteristics as well as the final beef qualities including pH decline, color and tenderness. To do so, genetic selection, farming system practices and characteristics of the animals before slaughter have to be considered to achieve the suitable goals.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Zierath, J. R.; Hawley, J. A., Skeletal muscle fiber type: influence on contractile and metabolic properties. *PLoS Biol* **2004**, *2*, e348.
2. Lefaucheur, L., A second look into fibre typing--relation to meat quality. *Meat science* **2010**, *84*, 257-70.
3. Ouali, A.; Gagaoua, M.; Boudida, Y.; Becila, S.; Boudjellal, A.; Herrera-Mendez, C. H.; Sentandreu, M. A., Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat science* **2013**, *95*, 854-70.
4. Listrat, A.; Lebret, B.; Louveau, I.; Astruc, T.; Bonnet, M.; Lefaucheur, L.; Picard, B.; Bugeon, J., How Muscle Structure and Composition Influence Meat and Flesh Quality. *TheScientificWorldJournal* **2016**, *2016*, 3182746.
5. Astruc, T.; Venien, A., Muscle fibre type and beef quality. In *Ensuring safety and quality in the production of beef*, Michael E. Dikeman: 2017; pp 1-23.

6. Choi, Y. M.; Kim, B. C., Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Science* **2009**, *122*, 105-118.
7. Chriki, S.; Picard, B.; Jurie, C.; Reichstadt, M.; Micol, D.; Brun, J. P.; Journaux, L.; Hocquette, J. F., Meta-analysis of the comparison of the metabolic and contractile characteristics of two bovine muscles: longissimus thoracis and semitendinosus. *Meat science* **2012**, *91*, 423-9.
8. Gagaoua, M.; Listrat, A.; Normand, J.; Andueza, D. J.; Gruffat, D.; Mairesse, G.; Chesneau, G.; Mourot, B. P.; Gobert, C.; Picard, B., Are there consistent relationships between major connective tissue components, intramuscular fat content and muscle fibre types in cattle muscle? *Animal* **2020**, 1-9.
9. Gil, M.; Delday, M. I.; Gispert, M.; MF, I. F.; Maltin, C. M.; Plastow, G. S.; Klont, R.; Sosnicki, A. A.; Carrion, D., Relationships between biochemical characteristics and meat quality of Longissimus thoracis and Semimembranosus muscles in five porcine lines. *Meat science* **2008**, *80*, 927-33.
10. Jurie, C.; Picard, B.; Geay, Y., Changes in the Metabolic and Contractile Characteristics of Muscle in Male Cattle Between 10 and 16 Months of Age. *The Histochemical Journal* **1999**, *31*, 117-122.
11. Kim, G. D., Analysis of Myosin Heavy Chain Isoforms from Longissimus Thoracis Muscle of Hanwoo Steer by Electrophoresis and LC-MS/MS. *Korean J Food Sci Anim Resour* **2014**, *34*, 656-64.
12. Kim, G. D.; Yang, H. S.; Jeong, J. Y., Comparison of Characteristics of Myosin Heavy Chain-based Fiber and Meat Quality among Four Bovine Skeletal Muscles. *Korean J Food Sci Anim Resour* **2016**, *36*, 819-828.
13. Dransfield, E.; Martin, J.-F.; Bauchart, D.; Abouelkaram, S.; Lepetit, J.; Culioli, J.; Jurie, C.; Picard, B., Meat quality and composition of three muscles from French cull cows and young bulls. *Animal Science* **2003**, *76*, 387-399.
14. Mouzo, D.; Rodríguez-Vázquez, R.; Lorenzo, J. M.; Franco, D.; Zapata, C.; López-Pedrouso, M., Proteomic application in predicting food quality relating to animal welfare. A review. *Trends in Food Science & Technology* **2020**, *99*, 520-530.
15. Picard, B.; Lefaucheur, L.; Berri, C.; Duclos, J. M., Muscle fibre ontogenesis in farm animal species. *Reprod. Nutr. Dev.* **2002**, *42*, 415-431.
16. Du, M.; Huang, Y.; Das, A. K.; Yang, Q.; Duarte, M. S.; Dodson, M. V.; Zhu, M. J., Meat Science and Muscle Biology Symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. *J Anim Sci* **2013**, *91*, 1419-27.
17. Du, M.; Wang, B.; Fu, X.; Yang, Q.; Zhu, M.-J., Fetal programming in meat production. *Meat science* **2015**, *109*, 40-47.
18. Gagaoua, M.; Hafid, K.; Boudida, Y.; Becila, S.; Ouali, A.; Picard, B.; Boudjellal, A.; Sentandreu, M. A., Caspases and Thrombin Activity Regulation by Specific Serpin Inhibitors in Bovine Skeletal Muscle. *Appl Biochem Biotechnol* **2015**, *177*, 279-303.
19. Ertbjerg, P.; Puolanne, E., Muscle structure, sarcomere length and influences on meat quality: A review. *Meat science* **2017**, *132*, 139-152.

897 20. Faulkner, G.; Lanfranchi, G.; Valle, G., Telethonin and other new proteins of the Z-disc
898 of skeletal muscle. *IUBMB Life* **2001**, *51*, 275-82.
899 21. Gontier, Y.; Taivainen, A.; Fontao, L.; Sonnenberg, A.; van der Flier, A.; Carpen, O.;
900 Faulkner, G.; Borradori, L., The Z-disc proteins myotilin and FATZ-1 interact with each other
901 and are connected to the sarcolemma via muscle-specific filamins. *Journal of cell science*
902 **2005**, *118*, 3739-3749.
903 22. Bottinelli, R.; Reggiani, C., Human skeletal muscle fibres: molecular and functional
904 diversity. *Progress in Biophysics and Molecular Biology* **2000**, *73*, 195-262.
905 23. Gagaoua, M.; Bonnet, M.; De Koning, L.; Picard, B., Reverse Phase Protein array for the
906 quantification and validation of protein biomarkers of beef qualities: The case of meat color
907 from Charolais breed. *Meat science* **2018**, *145*, 308-319.
908 24. Gagaoua, M.; Bonnet, M.; Ellies-Oury, M. P.; De Koning, L.; Picard, B., Reverse phase
909 protein arrays for the identification/validation of biomarkers of beef texture and their use for
910 early classification of carcasses. *Food Chemistry* **2018**, *250*, 245-252.
911 25. Picard, B.; Gagaoua, M., Chapter 11 - Proteomic Investigations of Beef Tenderness. In
912 *Proteomics in Food Science: from farm to fork*, Colgrave, M. L., Ed. Academic Press:
913 London, 2017; pp 177-197.
914 26. Picard, B.; Gagaoua, M., Meta-proteomics for the discovery of protein biomarkers of beef
915 tenderness: An overview of integrated studies. *Food Res Int* **2020**, *127*, 108739.
916 27. Schiaffino, S.; Reggiani, C., Fiber types in mammalian skeletal muscles. *Physiol Rev*
917 **2011**, *91*, 1447-531.
918 28. England, E. M.; Matarneh, S. K.; Mitacek, R. M.; Abraham, A.; Ramanathan, R.; Wicks,
919 J. C.; Shi, H.; Scheffler, T. L.; Oliver, E. M.; Helm, E. T.; Gerrard, D. E., Presence of oxygen
920 and mitochondria in skeletal muscle early postmortem. *Meat science* **2018**, *139*, 97-106.
921 29. Picard, B.; Duris, M. P.; Jurie, C., Classification of Bovine Muscle Fibres by Different
922 Histochemical Techniques. *The Histochemical Journal* **1998**, *30*, 473-477.
923 30. Picard, B.; Jurie, C.; Duris, M. P.; Renand, G., Consequences of selection for higher
924 growth rate on muscle fibre development in cattle. *Livestock Science* **2006**, *102*, 107-120.
925 31. Gagaoua, M.; Monteils, V.; Couvreur, S.; Picard, B., Identification of Biomarkers
926 Associated with the Rearing Practices, Carcass Characteristics, and Beef Quality: An
927 Integrative Approach. *Journal of Agricultural and Food Chemistry* **2017**, *65*, 8264-8278.
928 32. Joo, S. T.; Kim, G. D.; Hwang, Y. H.; Ryu, Y. C., Control of fresh meat quality through
929 manipulation of muscle fiber characteristics. *Meat science* **2013**, *95*, 828-36.
930 33. Maltin, C. A.; Sinclair, K. D.; Warriss, P. D.; Grant, C. M.; Porter, A. D.; Delday, M. I.;
931 Warkup, C. C., The effects of age at slaughter, genotype and finishing system on the
932 biochemical properties, muscle fibre type characteristics and eating quality of bull beef from
933 suckled calves. *Animal Science* **1998**, *66*, 341-348.
934 34. Oury, M. P.; Dumont, R.; Jurie, C.; Hocquette, J. F.; Picard, B., Specific fibre
935 composition and metabolism of the rectus abdominis muscle of bovine Charolais cattle. *BMC*
936 *Biochem* **2010**, *11*, 12.
937 35. Costagliola, A.; Wojcik, S.; Pagano, T. B.; De Biase, D.; Russo, V.; Iovane, V.; Grieco,
938 E.; Papparella, S.; Paciello, O., Age-Related Changes in Skeletal Muscle of Cattle. *Veterinary*
939 *Pathology* **2016**, *53*, 436-446.

940 36. Meunier, B.; Picard, B.; Astruc, T.; Labas, R., Development of image analysis tool for the
941 classification of muscle fibre type using immunohistochemical staining. *Histochemistry and*
942 *Cell Biology* **2010**, *134*, 307-317.
943 37. Astruc, T.; Peyrin, F.; Vénien, A.; Labas, R.; Abrantes, M.; Dumas, P.; Jamme, F., In situ
944 thermal denaturation of myofibre sub-type proteins studied by immunohistofluorescence and
945 synchrotron radiation FT-IR microspectroscopy. *Food Chemistry* **2012**, *134*, 1044-1051.
946 38. Picard, B.; Leger, J.; Robelin, J., Quantitative determination of type I myosin heavy chain
947 in bovine muscle with anti myosin monoclonal antibodies. *Meat science* **1994**, *36*, 333-43.
948 39. Picard, B.; Barboiron, C.; Duris, M. P.; Gagniere, H.; Jurie, C.; Geay, Y., Electrophoretic
949 separation of bovine muscle myosin heavy chain isoforms. *Meat science* **1999**, *53*, 1-7.
950 40. Picard, B.; Barboiron, C.; Chadeyron, D.; Jurie, C., Protocol for high-resolution
951 electrophoresis separation of myosin heavy chain isoforms in bovine skeletal muscle.
952 *Electrophoresis* **2011**, *32*, 1804-1806.
953 41. Scheffler, T. L.; Leitner, M. B.; Wright, S. A., Technical note: Protocol for
954 electrophoretic separation of bovine myosin heavy chain isoforms and comparison to
955 immunohistochemistry analysis. *J Anim Sci* **2018**, *96*, 4306-4312.
956 42. Guth, L.; Samaha, F. J., Procedure for the histochemical demonstration of actomyosin
957 ATPase. *Experimental Neurology* **1970**, *28*, 365-367.
958 43. Brooke, M. H.; Kaiser, K. K., Muscle fiber types: How many and what kind? *Archives of*
959 *Neurology* **1970**, *23*, 369-379.
960 44. Ashmore, C. R.; Vigneron, P.; Marger, L.; Doerr, L., Simultaneous cytochemical
961 demonstration of muscle fiber types and acetylcholinesterase in muscle fibers of dystrophic
962 chickens. *Exp Neurol* **1978**, *60*, 68-82.
963 45. Peter, J. B.; Barnard, R. J.; Edgerton, V. R.; Gillespie, C. A.; Stempel, K. E., Metabolic
964 profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **1972**,
965 *11*, 2627-2633.
966 46. Astruc, T.; Loison, O.; Jamme, F.; Réfrégiers, M.; Vénien, A., Preferred metabolic
967 pathway of bovine muscle fibre revealed by synchrotron–deep ultraviolet fluorescence
968 imaging. *Journal of Spectral Imaging* **2019**, *8*, a1.
969 47. Wen, Y.; Murach, K. A.; Jr., I. J. V.; Fry, C. S.; Vickery, C.; Peterson, C. A.; McCarthy,
970 J. J.; Campbell, K. S., MyoVision: software for automated high-content analysis of skeletal
971 muscle immunohistochemistry. *Journal of Applied Physiology* **2018**, *124*, 40-51.
972 48. Kammoun, M.; Cassar-Malek, I.; Meunier, B.; Picard, B., A simplified
973 immunohistochemical classification of skeletal muscle fibres in mouse. *European Journal of*
974 *Histochemistry* **2014**, *58*.
975 49. Hemmings, K. M.; Parr, T.; Daniel, Z. C. T. R.; Picard, B.; Buttery, P. J.; Brameld, J. M.,
976 Examination of myosin heavy chain isoform expression in ovine skeletal muscles1. *Journal of*
977 *Animal Science* **2009**, *87*, 3915-3922.
978 50. Yates, D. T.; Cadaret, C. N.; Beede, K. A.; Riley, H. E.; Macko, A. R.; Anderson, M. J.;
979 Camacho, L. E.; Limesand, S. W., Intrauterine growth-restricted sheep fetuses exhibit smaller
980 hindlimb muscle fibers and lower proportions of insulin-sensitive Type I fibers near term.
981 *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **2016**,
982 *310*, R1020-R1029.

983 51. Hamdi, H.; Majdoub-Mathlouthi, L.; Picard, B.; Listrat, A.; Durand, D.; Znaïdi, I. A.;
984 Kraiem, K., Carcass traits, contractile muscle properties and meat quality of grazing and
985 feedlot Barbarine lamb receiving or not olive cake. *Small Ruminant Research* **2016**, *145*,
986 85-93.
987 52. Abdelhadi, O. M.; Babiker, S. A.; Picard, B.; Jurie, C.; Jailler, R.; Hocquette, J. F.; Faye,
988 B., Effect of season on contractile and metabolic properties of desert camel muscle (Camelus
989 dromedarius). *Meat science* **2012**, *90*, 139-44.
990 53. Al-Owaimer, A. N.; Suliman, G. M.; Sami, A. S.; Picard, B.; Hocquette, J. F., Chemical
991 composition and structural characteristics of Arabian camel (Camelus dromedarius) m.
992 longissimus thoracis. *Meat science* **2014**, *96*, 1233-41.
993 54. Picard, B.; Cassar-Malek, I., Evidence for expression of IIb myosin heavy chain isoform
994 in some skeletal muscles of Blonde d'Aquitaine bulls. *Meat science* **2009**, *82*, 30-6.
995 55. Maccatrozzo, L.; Patruno, M.; Toniolo, L.; Reggiani, C.; Mascarello, F., Myosin Heavy
996 Chain 2B isoform is expressed in specialized eye muscles but not in trunk and limb muscles
997 of cattle. *European Journal of Histochemistry* **2009**, *48*.
998 56. Chikuni, K.; Muroya, S.; Nakajima, I., Myosin heavy chain isoforms expressed in bovine
999 skeletal muscles. *Meat science* **2004**, *67*, 87-94.
1000 57. Picard, B.; Allais, S.; Jurie, C.; Cassar-Malek, I.; Levéziel, H.; Journaux, L.; Renand, G.
1001 In *A particular myosin heavy chain isoform variable between beef breeds*, Proceedings of the
1002 61st Annual Meeting of the European Association for Animal Production (EAAP), Heraklion,
1003 2010; 2010.
1004 58. Moran, L.; O'Sullivan, M. G.; Kerry, J. P.; Picard, B.; McGee, M.; O'Riordan, E. G.;
1005 Moloney, A. P., Effect of a grazing period prior to finishing on a high concentrate diet on
1006 meat quality from bulls and steers. *Meat science* **2017**, *125*, 76-83.
1007 59. Gagaoua, M.; Terlouw, E. M. C.; Micol, D.; Hocquette, J. F.; Moloney, A. P.; Nuernberg,
1008 K.; Bauchart, D.; Boudjellal, A.; Scollan, N. D.; Richardson, R. I.; Picard, B., Sensory quality
1009 of meat from eight different types of cattle in relation with their biochemical characteristics.
1010 *Journal of Integrative Agriculture* **2016**, *15*, 1550-1563.
1011 60. Gagaoua, M.; Terlouw, E. M.; Boudjellal, A.; Picard, B., Coherent correlation networks
1012 among protein biomarkers of beef tenderness: What they reveal. *J Proteomics* **2015**, *128*,
1013 365-74.
1014 61. Talmadge, R. J.; Roy, R. R., Electrophoretic separation of rat skeletal muscle myosin
1015 heavy-chain isoforms. *Journal of Applied Physiology* **1993**, *75*, 2337-2340.
1016 62. Oury, M. P.; Picard, B.; Briand, M.; Blanquet, J. P.; Dumont, R., Interrelationships
1017 between meat quality traits, texture measurements and physicochemical characteristics of M.
1018 rectus abdominis from Charolais heifers. *Meat science* **2009**, *83*, 293-301.
1019 63. Guillemin, N.; Meunier, B.; Jurie, C.; Cassar-Malek, I.; Hocquette, J. F.; Leveziel, H.;
1020 Picard, B., Validation of a Dot-Blot quantitative technique for large scale analysis of beef
1021 tenderness biomarkers. *J Physiol Pharmacol* **2009**, *60 Suppl 3*, 91-7.
1022 64. Gagaoua, M.; Monteils, V.; Picard, B., Data from the farmgate-to-meat continuum
1023 including omics-based biomarkers to better understand the variability of beef tenderness: An
1024 integromics approach. *J Agric Food Chem* **2018**, *66*, 13552–13563.

65. Picard, B.; Gagaoua, M.; Al-Jammas, M.; De Koning, L.; Valais, A.; Bonnet, M., Beef tenderness and intramuscular fat proteomic biomarkers: muscle type effect. *PeerJ* **2018**, *6*, e4891.
66. Picard, B.; Gagaoua, M.; Al Jammas, M.; Bonnet, M., Beef tenderness and intramuscular fat proteomic biomarkers: Effect of gender and rearing practices. *J Proteomics* **2019**, *200*, 1-10.
67. Hocquette, J.-F.; Jurie, C.; Picard, B.; Alberti, P.; Panea, B.; Christensen, M.; Failla, S.; Gigli, S.; Levéziel, H.; Olleta, J. L.; Sañudo, C.; Ertbjerg, P.; Nute, G. R.; Williams, J. L., Metabolic and contractile characteristics of Longissimus thoracis muscle of young bulls from 15 European breeds in relationship with body composition. In *2. International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2007)*, Wageningen Academic Publishers: Vichy, 2007; pp 111-112.
68. Jurie, C.; Ortigues-Marty, I.; Picard, B.; Micol, D.; Hocquette, J. F., The separate effects of the nature of diet and grazing mobility on metabolic potential of muscles from Charolais steers. *Livestock Science* **2006**, *104*, 182-192.
69. Schreurs, N. M.; Garcia, F.; Jurie, C.; Agabriel, J.; Micol, D.; Bauchart, D.; Listrat, A.; Picard, B., Meta-analysis of the effect of animal maturity on muscle characteristics in different muscles, breeds, and sexes of cattle. *Journal of Animal Science* **2008**, *86*, 2872-2887.
70. Talmant, A.; Monin, G.; Briand, M.; Dadet, M.; Briand, Y., Activities of metabolic and contractile enzymes in 18 bovine muscles. *Meat science* **1986**, *18*, 23-40.
71. Gagaoua, M.; Terlouw, E. M. C.; Picard, B., The study of protein biomarkers to understand the biochemical processes underlying beef color development in young bulls. *Meat science* **2017**, *134*, 18-27.
72. Totland, G. K.; Kryvi, H., Distribution patterns of muscle fibre types in major muscles of the bull (Bos taurus). *Anatomy and Embryology* **1991**, *184*, 441-450.
73. Rosser, B. W.; Norris, B. J.; Nemeth, P. M., Metabolic capacity of individual muscle fibers from different anatomic locations. *Journal of Histochemistry & Cytochemistry* **1992**, *40*, 819-825.
74. Kirchofer, K. S.; Calkins, C. R.; Gwartney, B. L., Fiber-type composition of muscles of the beef chuck and round1. *Journal of Animal Science* **2002**, *80*, 2872-2878.
75. Brandstetter, A. M.; Picard, B.; Geay, Y., Regional variations of muscle fibre characteristics in m. semitendinosus of growing cattle. *Journal of Muscle Research & Cell Motility* **1997**, *18*, 57-62.
76. Hunt, M. C.; Hedrick, H. B., Profile of Fiber Types and Related Properties of Five Bovine Muscles. *Journal of Food Science* **1977**, *42*, 513-517.
77. Van Bibber-Krueger, C. L.; Collins, A. M.; Phelps, K. J.; O'Quinn, T. G.; Houser, T. A.; Turner, K. K.; Gonzalez, J. M., Effects of quality grade and intramuscular location on beef semitendinosus muscle fiber characteristics, NADH content, and color stability. *J Anim Sci* **2020**, *98*.
78. Picard, B.; Gagnière, H.; Geay, Y., Contractile differentiation of bovine maseter muscle. *BAM-PADOVA*- **1996**, *6*, 361-372.

79. Santé-Lhoutellier, V.; Gatellier, P.; Fiot, I.; Durand, D.; Micol, D.; Picard, B., Specific features of muscles and meat from 'AOC' guaranteed-origin Taureau de Camargue beef cattle. *Livestock Science* **2010**, *129*, 31-37.
80. Couvreur, S.; Le Bec, G.; Micol, D.; Picard, B., Relationships Between Cull Beef Cow Characteristics, Finishing Practices and Meat Quality Traits of Longissimus thoracis and Rectus abdominis. *Foods* **2019**, *8*, 141.
81. Gagaoua, M.; Couvreur, S.; Le Bec, G.; Aminot, G.; Picard, B., Associations among Protein Biomarkers and pH and Color Traits in Longissimus thoracis and Rectus abdominis Muscles in Protected Designation of Origin Maine-Anjou Cull Cows. *J Agric Food Chem* **2017**, *65*, 3569-3580.
82. Bonnet, M.; Cassar-Malek, I.; Chilliard, Y.; Picard, B., Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. *animal* **2010**, *4*, 1093-1109.
83. Picard, B.; Berri, C.; Lefaucheur, L.; Molette, C.; Sayd, T.; Terlouw, C., Skeletal muscle proteomics in livestock production. *Brief Funct Genomics* **2010**, *9*, 259-78.
84. Chen, D.; Li, W.; Du, M.; Cao, B., Adipogenesis, fibrogenesis and myogenesis related gene expression in longissimus muscle of high and low marbling beef cattle. *Livestock Science* **2019**, *229*, 188-193.
85. Pearson, A. M., Muscle growth and exercise. *Critical Reviews in Food Science and Nutrition* **1990**, *29*, 167-196.
86. Shrager, J. B.; Desjardins, P. R.; Burkman, J. M.; Konig, S. K.; Stewart, D. R.; Su, L.; Shah, M. C.; Bricklin, E.; Tewari, M.; Hoffman, R.; Rickels, M. R.; Jullian, E. H.; Rubinstein, N. A.; Stedman, H. H., Human skeletal myosin heavy chain genes are tightly linked in the order embryonic-IIa-IId/x-IIb-perinatal-extraocular. *Journal of Muscle Research & Cell Motility* **2000**, *21*, 345-355.
87. Maier, A.; McEwan, J. C.; Dodds, K. G.; Fischman, D. A.; Fitzsimons, R. B.; Harris, A. J., Myosin heavy chain composition of single fibres and their origins and distribution in developing fascicles of sheep tibialis cranialis muscles. *Journal of Muscle Research & Cell Motility* **1992**, *13*, 551-572.
88. Cho, M.; Hughes, S. M.; Karsch-Mizrachi, I.; Travis, M.; Leinwand, L. A.; Blau, H. M., Fast myosin heavy chains expressed in secondary mammalian muscle fibers at the time of their inception. *Journal of cell science* **1994**, *107*, 2361-2371.
89. Chaze, T.; Meunier, B.; Chambon, C.; Jurie, C.; Picard, B., In vivo proteome dynamics during early bovine myogenesis. *Proteomics* **2008**, *8*, 4236-48.
90. Chaze, T.; Meunier, B.; Chambon, C.; Jurie, C.; Picard, B., Proteome dynamics during contractile and metabolic differentiation of bovine foetal muscle. *Animal* **2009**, *3*, 980-1000.
91. Brameld, J. M.; Daniel, Z. C. T. R., In utero effects on livestock muscle development and body composition. *Australian Journal of Experimental Agriculture* **2008**, *48*, 921-929.
92. Biressi, S.; Molinaro, M.; Cossu, G., Cellular heterogeneity during vertebrate skeletal muscle development. *Developmental Biology* **2007**, *308*, 281-293.
93. Greenwood, P. L.; Slepetis, R. M.; Bell, A. W.; Hermanson, J. W., Intrauterine growth retardation is associated with reduced cell cycle activity, but not myofibre number, in ovine fetal muscle. *Reproduction, Fertility and Development* **2000**, *11*, 281-291.

1110 94. Greenwood, P. L.; Slepetis, R. M.; Bell, A. W., Influences on fetal and placental weights
1111 during mid to late gestation in prolific ewes well nourished throughout pregnancy.
1112 *Reproduction, Fertility and Development* **2001**, *12*, 149-156.
1113 95. Zhu, M. J.; Ford, S. P.; Means, W. J.; Hess, B. W.; Nathanielsz, P. W.; Du, M., Maternal
1114 nutrient restriction affects properties of skeletal muscle in offspring. *The Journal of*
1115 *Physiology* **2006**, *575*, 241-250.
1116 96. Fu, X.; Yang, Q.; Wang, B.; Zhao, J.; Zhu, M.; Parish, S. M.; Du, M., Reduced satellite
1117 cell density and myogenesis in Wagyu compared with Angus cattle as a possible explanation
1118 of its high marbling. *animal* **2017**, *12*, 990-997.
1119 97. Lefaucheur, L.; Gerrard, D., Muscle fiber plasticity in farm mammals. *Journal of Animal*
1120 *Science* **2000**, *77*, 1-19.
1121 98. Talbot, J.; Maves, L., Skeletal muscle fiber type: using insights from muscle
1122 developmental biology to dissect targets for susceptibility and resistance to muscle disease.
1123 *WIREs Developmental Biology* **2016**, *5*, 518-534.
1124 99. Schiaffino, S.; Sandri, M.; Murgia, M., Activity-Dependent Signaling Pathways
1125 Controlling Muscle Diversity and Plasticity. *Physiology* **2007**, *22*, 269-278.
1126 100. Polla, B.; D'Antona, G.; Bottinelli, R.; Reggiani, C., Respiratory muscle fibres:
1127 specialisation and plasticity. *Thorax* **2004**, *59*, 808-817.
1128 101. Woo, M.; Isganaitis, E.; Cerletti, M.; Fitzpatrick, C.; Wagers, A. J.;
1129 Jimenez-Chillaron, J.; Patti, M. E., Early life nutrition modulates muscle stem cell number:
1130 implications for muscle mass and repair. *Stem Cells Dev* **2011**, *20*, 1763-9.
1131 102. Ward, A.; Crouse, M.; Cushman, R.; McLean, K.; Dahlen, C.; Borowicz, P.;
1132 Reynolds, L.; Caton, J., Maternal nutrient restriction in early gestation upregulates myogenic
1133 genes in cattle fetal muscle tissue. *International Society of Animal Genetics* **2017**.
1134 103. Kaspric, N.; Picard, B.; Reichstadt, M.; Tournayre, J.; Bonnet, M., ProteINSIDE to
1135 Easily Investigate Proteomics Data from Ruminants: Application to Mine Proteome of
1136 Adipose and Muscle Tissues in Bovine Foetuses. *PLoS One* **2015**, *10*, e0128086.
1137 104. Bonnet, M.; Kaspric, N.; Vonnahme, K.; Barboiron, C.; Viala, D.; Chadeyron, D.;
1138 Picard, B., Maternal nutrient restriction followed by realimentation from early to
1139 mid-gestation in beef cows impacts proteomes of fetal muscular and adipose tissues. *ICoMST,*
1140 *Clermont-Ferrand* **2015**, *111*.
1141 105. Deng, B.; Zhang, F.; Wen, J.; Ye, S.; Wang, L.; Yang, Y.; Gong, P.; Jiang, S., The
1142 function of myostatin in the regulation of fat mass in mammals. *Nutrition & Metabolism*
1143 **2017**, *14*, 29.
1144 106. Rodriguez, J.; Vernus, B.; Chelh, I.; Cassar-Malek, I.; Gabillard, J. C.; Hadj Sassi,
1145 A.; Seiliez, I.; Picard, B.; Bonnieu, A., Myostatin and the skeletal muscle atrophy and
1146 hypertrophy signaling pathways. *Cellular and Molecular Life Sciences* **2014**, *71*, 4361-4371.
1147 107. Wang, M.; Yu, H.; Kim, Y. S.; Bidwell, C. A.; Kuang, S., Myostatin facilitates slow
1148 and inhibits fast myosin heavy chain expression during myogenic differentiation. *Biochemical*
1149 *and Biophysical Research Communications* **2012**, *426*, 83-88.
1150 108. McPherron, A. C.; Lee, S.-J., Double muscling in cattle due to mutations in the
1151 myostatin gene. *Proceedings of the National Academy of Sciences* **1997**, *94*, 12457-12461.

1152 109. Fiems, L. O., Double Muscling in Cattle: Genes, Husbandry, Carcasses and Meat.
1153 *Animals* **2012**, *2*, 472-506.
1154 110. Deveaux, V.; Cassar-Malek, I.; Picard, B., Comparison of contractile characteristics
1155 of muscle from Holstein and double-muscled Belgian Blue foetuses. *Comp Biochem Physiol*
1156 *A Mol Integr Physiol* **2001**, *131*, 21-9.
1157 111. Bouley, J.; Meunier, B.; Chambon, C.; De Smet, S.; Hocquette, J. F.; Picard, B.,
1158 Proteomic analysis of bovine skeletal muscle hypertrophy. *Proteomics* **2005**, *5*, 490-500.
1159 112. Mouton, J.; Loos, B.; Moolman-Smook, J. C.; Kinnear, C. J., Ascribing novel
1160 functions to the sarcomeric protein, myosin binding protein H (MyBPH) in cardiac sarcomere
1161 contraction. *Experimental Cell Research* **2015**, *331*, 338-351.
1162 113. Listrat, A.; Picard, B.; JAILLER, R.; Collignon, H.; Peccatte, J.-R.; Micol, D.; Geay,
1163 Y.; Dozias, D., Grass valorisation and muscular characteristics of blonde d'Aquitaine steers.
1164 *Anim. Res.* **2001**, *50*, 105-118.
1165 114. Renand, G.; Jurie, C.; Robelin, J.; Picard, B.; Geay, Y.; Ménissier, F., Genetic
1166 variability of muscle biological characteristics of young Limousin bulls. *Genetics Selection*
1167 *Evolution* **1995**, *27*, 287.
1168 115. Duris, M. P.; Renand, G.; Picard, B., Genetic Variability of Foetal Bovine Myoblasts
1169 in Primary Culture. *The Histochemical Journal* **1999**, *31*, 753-760.
1170 116. Wegner, J.; Albrecht, E.; Fiedler, I.; Teuscher, F.; Papstein, H. J.; Ender, K., Growth-
1171 and breed-related changes of muscle fiber characteristics in cattle. *J Anim Sci* **2000**, *78*,
1172 1485-96.
1173 117. Jiang, H.; Ge, X., Meat Science and Muscle Biology Symposium--mechanism of
1174 growth hormone stimulation of skeletal muscle growth in cattle. *J Anim Sci* **2014**, *92*, 21-9.
1175 118. Brandstetter, A. M.; Picard, B.; Geay, Y., Muscle fibre characteristics in four
1176 muscles of growing bulls: I. Postnatal differentiation. *Livestock Production Science* **1998**, *53*,
1177 15-23.
1178 119. Picard, B.; Robelin, J.; Geay, Y., Influence of castration and postnatal energy
1179 restriction on the contractile and metabolic characteristics of bovine muscle. *Ann. Zootech.*
1180 **1995**, *44*, 347-357.
1181 120. Young, O. A.; Bass, J. J., Effect of castration on bovine muscle composition. *Meat*
1182 *science* **1984**, *11*, 139-156.
1183 121. Johnston, D. M.; Moody, W. G.; Boling, J. A.; Bradley, N. W., Influence of Breed
1184 Type, Sex, Feeding Systems, and Muscle Bundle Size on Bovine Fiber Type Characteristics.
1185 *Journal of Food Science* **1981**, *46*, 1760-1765.
1186 122. Vestergaard, M.; Henckel, P.; Oksbjerg, N.; Sejrsen, K., The effect of cimaterol on
1187 muscle fiber characteristics, capillary supply, and metabolic potentials of longissimus and
1188 semitendinosus muscles from young Friesian bulls. *J Anim Sci* **1994**, *72*, 2298-306.
1189 123. Gonzalez, J. M.; Dijkhuis, R. D.; Johnson, D. D.; Carter, J. N.; Johnson, S. E.,
1190 Differential response of cull cow muscles to the hypertrophic actions of
1191 ractopamine-hydrogen chloride1. *Journal of Animal Science* **2008**, *86*, 3568-3574.
1192 124. Gonzalez, J. M.; Carter, J. N.; Johnson, D. D.; Ouellette, S. E.; Johnson, S. E., Effect
1193 of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area,

1194 diameter, and satellite cell numbers in cull beef cows1. *Journal of Animal Science* **2007**, *85*,
1195 1893-1901.
1196 125. Wicks, J.; Beline, M.; Gomez, J. F. M.; Luzardo, S.; Silva, S. L.; Gerrard, D., Muscle
1197 Energy Metabolism, Growth, and Meat Quality in Beef Cattle. *Agriculture* **2019**, *9*, 195.
1198 126. Maltin, C. A.; Lobley, G. E.; Grant, C. M.; Miller, L. A.; Kyle, D. J.; Horgan, G. W.;
1199 Matthews, K. R.; Sinclair, K. D., Factors influencing beef eating quality 2. Effects of
1200 nutritional regimen and genotype on muscle fibre characteristics. *Animal Science* **2016**, *72*,
1201 279-287.
1202 127. Cassar-Malek, I.; Hocquette, J.; Jurie, C.; Listrat, A.; Jailler, R.; Bauchart, D.;
1203 Briand, Y.; Picard, B., Muscle-specific metabolic, histochemical and biochemical responses
1204 to a nutritionally induced discontinuous growth path. *Animal science* **2004**, *79*, 49-59.
1205 128. Hornick, J. L.; Van Eenaeme, C.; Gérard, O.; Dufrasne, I.; Istasse, L., Mechanisms
1206 of reduced and compensatory growth. *Domestic Animal Endocrinology* **2000**, *19*, 121-132.
1207 129. Oddy, V. H.; Harper, G. S.; Greenwood, P. L.; McDonagh, M. B., Nutritional and
1208 developmental effects on the intrinsic properties of muscles as they relate to the eating quality
1209 of beef. *Australian Journal of Experimental Agriculture* **2001**, *41*, 921.
1210 130. Yambayamba, E.; Price, M. A., Fiber-type proportions and diameters in the
1211 longissimus muscle of beef heifers undergoing catch-up (compensatory) growth. *Canadian*
1212 *Journal of Animal Science* **1991**, *71*, 1031-1035.
1213 131. Tomkins, N. W.; Harper, G. S.; Bruce, H. L.; Hunter, R. A., Effect of different
1214 post-weaning growth paths on long-term weight gain, carcass characteristics and eating
1215 quality of beef cattle. *Australian Journal of Experimental Agriculture* **2006**, *46*, 1571-1578.
1216 132. Vestergaard, M.; Oksbjerg, N.; Henckel, P., Influence of feeding intensity, grazing
1217 and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus,
1218 longissimus dorsi and supraspinatus muscles of young bulls. *Meat science* **2000**, *54*, 177-85.
1219 133. Dunne, P. G.; Keane, M. G.; O'Mara, F. P.; Monahan, F. J.; Moloney, A. P., Colour
1220 of subcutaneous adipose tissue and M. longissimus dorsi of high index dairy and beefxdairy
1221 cattle slaughtered at two liveweights as bulls and steers. *Meat science* **2004**, *68*, 97-106.
1222 134. White, A.; O'Sullivan, A.; Troy, D. J.; O'Neill, E. E., Manipulation of the pre-rigor
1223 glycolytic behaviour of bovine M. longissimus dorsi in order to identify causes of
1224 inconsistencies in tenderness. *Meat science* **2006**, *73*, 151-156.
1225 135. Hughes, J. M.; Clarke, F. M.; Purslow, P. P.; Warner, R. D., Meat color is
1226 determined not only by chromatic heme pigments but also by the physical structure and
1227 achromatic light scattering properties of the muscle. *Comprehensive Reviews in Food Science*
1228 *and Food Safety* **2020**, *19*, 44-63.
1229 136. Purslow, P. P.; Warner, R. D.; Clarke, F. M.; Hughes, J. M., Variations in meat
1230 colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited
1231 review). *Meat science* **2020**, *159*, 107941.
1232 137. Whipple, G.; Koohmaraie, M.; Dikeman, M. E.; Crouse, J. D., Predicting
1233 beef-longissimus tenderness from various biochemical and histological muscle traits2.
1234 *Journal of Animal Science* **1990**, *68*, 4193-4199.

1235 138. Ruusunen, M.; Puolanne, E., Histochemical properties of fibre types in muscles of
1236 wild and domestic pigs and the effect of growth rate on muscle fibre properties. *Meat science*
1237 **2004**, *67*, 533-539.
1238 139. Choe, J. H.; Choi, Y. M.; Lee, S. H.; Shin, H. G.; Ryu, Y. C.; Hong, K. C.; Kim, B.
1239 C., The relation between glycogen, lactate content and muscle fiber type composition, and
1240 their influence on postmortem glycolytic rate and pork quality. *Meat science* **2008**, *80*,
1241 355-362.
1242 140. Lee, S. H.; Joo, S. T.; Ryu, Y. C., Skeletal muscle fiber type and myofibrillar
1243 proteins in relation to meat quality. *Meat science* **2010**, *86*, 166-70.
1244 141. Ozawa, S.; Mitsuhashi, T.; Mitsumoto, M.; Matsumoto, S.; Itoh, N.; Itagaki, K.;
1245 Kohno, Y.; Dohgo, T., The characteristics of muscle fiber types of longissimus thoracis
1246 muscle and their influences on the quantity and quality of meat from Japanese Black steers.
1247 *Meat science* **2000**, *54*, 65-70.
1248 142. Ryu, Y. C.; Kim, B. C., The relationship between muscle fiber characteristics,
1249 postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. *Meat science*
1250 **2005**, *71*, 351-7.
1251 143. Carpenter, C. E.; Cornforth, D. P.; Whittier, D., Consumer preferences for beef color
1252 and packaging did not affect eating satisfaction. *Meat science* **2001**, *57*, 359-363.
1253 144. Ramanathan, R.; Mancini, R. A., Role of Mitochondria in Beef Color: A Review.
1254 *Meat and Muscle Biology* **2018**, *2*, 309-320.
1255 145. Klont, R. E.; Brocks, L.; Eikelenboom, G., Muscle fibre type and meat quality. *Meat*
1256 *science* **1998**, *49S1*, S219-29.
1257 146. Wu, S.; Luo, X.; Yang, X.; Hopkins, D. L.; Mao, Y.; Zhang, Y., Understanding the
1258 development of color and color stability of dark cutting beef based on mitochondrial
1259 proteomics. *Meat science* **2020**, *163*, 108046.
1260 147. Gagaoua, M.; Hughes, J.; Terlouw, C.; Warner, R.; Purslow, P.; Lorenzo, J. M.;
1261 Picard, B., Proteomic biomarkers of beef colour. *Trends in Food Science & Technology* **2020**,
1262 In press.
1263 148. Hughes, J.; Oiseth, S. K.; Purslow, P. P.; Warner, R. D., A structural approach to
1264 understanding the interactions between colour, water-holding capacity and tenderness. *Meat*
1265 *science* **2014**, *98*, 520-32.
1266 149. Hughes, J.; Clarke, F.; Purslow, P.; Warner, R., High pH in beef longissimus thoracis
1267 reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark
1268 colour. *Food Res Int* **2017**, *101*, 228-238.
1269 150. Mitacek, R. M.; Ke, Y.; Prenni, J. E.; Jadeja, R.; VanOverbeke, D. L.; Mafi, G. G.;
1270 Ramanathan, R., Mitochondrial Degeneration, Depletion of NADH, and Oxidative Stress
1271 Decrease Color Stability of Wet-Aged Beef Longissimus Steaks. *J Food Sci* **2019**, *84*, 38-50.
1272 151. McKeith, R. O.; King, D. A.; Grayson, A. L.; Shackelford, S. D.; Savell, J. W.;
1273 Wheeler, T. L., Influence of mitochondrial efficiency on beef lean color stability. *Meat*
1274 *science* **2014**, *96*, 476.
1275 152. Jeong, J. Y.; Hur, S. J.; Yang, H. S.; Moon, S. H.; Hwang, Y. H.; Park, G. B.; Joo, S.
1276 T., Discoloration characteristics of 3 major muscles from cattle during cold storage. *J Food*
1277 *Sci* **2009**, *74*, C1-5.

153. Tang, J.; Faustman, C.; Hoagland, T. A.; Mancini, R. A.; Seyfert, M.; Hunt, M. C., Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. *J Agric Food Chem* **2005**, *53*, 1223-30.
154. Renerre, M.; Labas, R., Biochemical factors influencing metmyoglobin formation in beef muscles. *Meat science* **1987**, *19*, 151-165.
155. Domínguez, R.; Pateiro, M.; Gagaoua, M.; Barba, F. J.; Zhang, W.; Lorenzo, J. M., A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants* **2019**, *8*, 429.
156. Faustman, C.; Cassens, R. G., The Biochemical Basis for Discoloration in Fresh Meat: A Review. *Journal of Muscle Foods* **1990**, *1*, 217-243.
157. Gagaoua, M.; Terlouw, E. M.; Micol, D.; Boudjellal, A.; Hocquette, J. F.; Picard, B., Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the Longissimus thoracis Muscle of Young Blond d'Aquitaine Bulls Using Protein Biomarkers. *J Agric Food Chem* **2015**, *63*, 6799-809.
158. Hailstones, D. L.; Gunning, P. W., Characterization of human myosin light chains 1sa and 3nm: implications for isoform evolution and function. *Molecular and Cellular Biology* **1990**, *10*, 1095-1104.
159. Dunne, P. G.; Monahan, F. J.; Moloney, A. P., Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? *Livestock Science* **2011**, *142*, 1-22.
160. Ponnampalam, E. N.; Hopkins, D. L.; Bruce, H.; Li, D.; Baldi, G.; Bekhit, A. E.-d., Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. *Comprehensive Reviews in Food Science and Food Safety* **2017**, *16*, 400-430.
161. Purchas, R. W.; Burnham, D. L.; Morris, S. T., Effects of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. *J Anim Sci* **2002**, *80*, 3211-21.
162. Sazili, A. Q.; Lee, G. K.; Parr, T.; Sensky, P. L.; Bardsley, R. G.; Buttery, P. J., The effect of altered growth rates on the calpain proteolytic system and meat tenderness in cattle. *Meat science* **2004**, *66*, 195-201.
163. Gagaoua, M.; Picard, B.; Monteils, V., Assessment of cattle inter-individual cluster variability: the potential of continuum data from the farm-to-fork for ultimate beef tenderness management. *J Sci Food Agric* **2019**, *99*, 4129-4141.
164. Gagaoua, M.; Picard, B., Current Advances in Meat Nutritional, Sensory and Physical Quality Improvement. *Foods* **2020**, *9*, 321.
165. Gagaoua, M.; Listrat, A.; Normand, J.; Gruffat, D.; Andueza, D.; Mairesse, G.; Mourot, B. P.; Chesneau, G.; Gobert, C.; Picard, B., Contribution of connective tissue components, muscle fibres and marbling to beef tenderness variability in longissimus thoracis, rectus abdominis, semimembranosus and semitendinosus muscles. *J Sci Food Agric* **2020**, *100*, 2502-2511.
166. Jurie, C.; Picard, B.; Hocquette, J. F.; Dransfield, E.; Micol, D.; Listrat, A., Muscle and meat quality characteristics of Holstein and Salers cull cows. *Meat science* **2007**, *77*, 459-66.

1321 167. Chardulo, L. A. L.; Baldassini, W. A.; Curi, R. A.; Pereira, G. L.; Machado Neto, O.
1322 R.; Dal-Pai, M.; Vechetti-Junior, I. J.; Malheiros, J. M.; Enriquez-Valencia, C. E., Gene and
1323 protein expression of myosin heavy chain in Nellore cattle comparing growth or meat
1324 tenderness traits. *Anim Biotechnol* **2019**, 1-10.
1325 168. Gagaoua, M.; Terlouw, C.; Richardson, I.; Hocquette, J. F.; Picard, B., The
1326 associations between proteomic biomarkers and beef tenderness depend on the end-point
1327 cooking temperature, the country origin of the panelists and breed. *Meat science* **2019**, *157*,
1328 107871.
1329 169. Maltin, C. A.; Balcerzak, D.; Tilley, R.; Delday, M., Determinants of meat quality:
1330 tenderness. *Proc Nutr Soc* **2003**, *62*, 337-47.
1331 170. Ouali, A., Meat Tenderization: Possible Causes and Mechanisms. A Review. *Journal*
1332 *of Muscle Foods* **1990**, *1*, 129-165.
1333 171. Zhao, C.; Zan, L.; Wang, Y.; Scott Updike, M.; Liu, G.; Bequette, B. J.; Baldwin Vi,
1334 R. L.; Song, J., Functional proteomic and interactome analysis of proteins associated with
1335 beef tenderness in Angus cattle. *Livestock Science* **2014**, *161*, 201-209.
1336 172. Grabez, V.; Kathri, M.; Phung, V.; Moe, K. M.; Slinde, E.; Skaugen, M.; Saarem, K.;
1337 Egelandsdal, B., Protein expression and oxygen consumption rate of early postmortem
1338 mitochondria relate to meat tenderness. *J Anim Sci* **2015**, *93*, 1967-79.
1339 173. Zapata, I.; Zerby, H. N.; Wick, M., Functional proteomic analysis predicts beef
1340 tenderness and the tenderness differential. *J Agric Food Chem* **2009**, *57*, 4956-63.
1341 174. Beldarrain, L. R.; Aldai, N.; Picard, B.; Sentandreu, E.; Navarro, J. L.; Sentandreu,
1342 M. A., Use of liquid isoelectric focusing (OFFGEL) on the discovery of meat tenderness
1343 biomarkers. *Journal of Proteomics* **2018**, *183*, 25-33.
1344 175. Picard, B.; Gagaoua, M.; Micol, D.; Cassar-Malek, I.; Hocquette, J. F.; Terlouw, C.
1345 E., Inverse relationships between biomarkers and beef tenderness according to contractile and
1346 metabolic properties of the muscle. *J Agric Food Chem* **2014**, *62*, 9808-18.
1347 176. Polati, R.; Menini, M.; Robotti, E.; Millioni, R.; Marengo, E.; Novelli, E.; Balzan, S.;
1348 Cecconi, D., Proteomic changes involved in tenderization of bovine Longissimus dorsi
1349 muscle during prolonged ageing. *Food Chemistry* **2012**, *135*, 2052-2069.
1350 177. Guillemin, N.; Bonnet, M.; Jurie, C.; Picard, B., Functional analysis of beef
1351 tenderness. *J Proteomics* **2011**, *75*, 352-65.
1352 178. Morzel, M.; Terlouw, C.; Chambon, C.; Micol, D.; Picard, B., Muscle proteome and
1353 meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: A central
1354 role of HSP27 isoforms. *Meat science* **2008**, *78*, 297-304.
1355 179. Rosa, A. F.; Moncau, C. T.; Poleti, M. D.; Fonseca, L. D.; Balieiro, J. C. C.; Silva, S.
1356 L. E.; Eler, J. P., Proteome changes of beef in Nellore cattle with different genotypes for
1357 tenderness. *Meat science* **2018**, *138*, 1-9.
1358 180. D'Alessandro, A.; Rinalducci, S.; Marrocco, C.; Zolla, V.; Napolitano, F.; Zolla, L.,
1359 Love me tender: an Omics window on the bovine meat tenderness network. *J Proteomics*
1360 **2012**, *75*, 4360-80.
1361 181. Carvalho, M. E.; Gasparin, G.; Poleti, M. D.; Rosa, A. F.; Balieiro, J. C.; Labate, C.
1362 A.; Nassu, R. T.; Tullio, R. R.; Regitano, L. C.; Mourao, G. B.; Coutinho, L. L., Heat shock

and structural proteins associated with meat tenderness in Nellore beef cattle, a Bos indicus breed. *Meat science* **2014**, *96*, 1318-24.

182. D'Alessandro, A.; Marrocco, C.; Rinalducci, S.; Mirasole, C.; Failla, S.; Zolla, L., Chianina beef tenderness investigated through integrated Omics. *J Proteomics* **2012**, *75*, 4381-98.

183. Bjarnadottir, S. G.; Hollung, K.; Hoy, M.; Bendixen, E.; Codrea, M. C.; Veiseth-Kent, E., Changes in protein abundance between tender and tough meat from bovine longissimus thoracis muscle assessed by isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and 2-dimensional gel electrophoresis analysis. *J Anim Sci* **2012**, *90*, 2035-43.

184. Malheiros, J. M.; Braga, C. P.; Grove, R. A.; Ribeiro, F. A.; Calkins, C. R.; Adamec, J.; Chardulo, L. A. L., Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat science* **2019**, *148*, 64-71.

Figure captions

Figure 1. ATPase activity of muscle fibers at different pHs from pH 4.2 to pH 4.4 (**a, b, c**) and succinate dehydrogenase (SHD) activity (**d**) according the protocols described in the literature [43-45].

(a) After incubation at pH 4.2, the ATPase activity of fast fibers is inhibited, only slow fibers are stained in black.

(b) After incubation at pH 4.3, the ATPase activity of fast fibers is differentially inhibited, slow fibers are stained in black and two shades of grey could be distinguished among the fast fibers.

(c) After an incubation at pH 4.4, only the ATPase activity of IIA fibers is inhibited, slow (I) and IIX fibers are stained in black

(d) Revelation of SDH activity, fibers with high SDH activity, so with great number of mitochondria, stained in blue.

The comparison the four serial sections allow to identify type I, IIA, IIX pure fibers and also IIC hybrid fibers containing both I and IIA MyHC.

Figure 2. The classification of muscle fibers using a combination of different anti-MyHC antibodies according to Picard *et al.* [29] and Meunier *et al.* [36].

(a) Antibody 5B9 from Agrobio (France) specific of MyHC I, labels slow type I fibers.

(b) Antibody 15F4 from Agrobio (France) specific of fast MyHC (IIa, IIx, IIb), labels all fast fibers (in white), slow fibers are in black.

(c) Antibody 8H2 from Agrobio (France) recognizes both IIx and I MyHC.

(d) The comparison of the 3 serial sections (**a**, **b**, **c**) using VISILOG software, allows to classify I, IIA, IIX pure fibers and fibers containing several MyHC isoforms, for example IIa/IIx, or slow/fast corresponding to IIC fibers described in the literature, not illustrated on the sections.

Figure 3. The separation of myosin heavy chain (MyHC) isoforms by electrophoresis.

(a) Separation of MyHC isoforms I, IIa, IIx, IIb of *Longissimus thoracis* (LT) muscle of 6 Blond d'Aquitaine young bulls 15 months-old, with (1, 2 3 and 4 wells) or without (5 and 6 wells) MyHC IIb, according to their molecular weights by the technique of Picard *et al.* [39]. The *Diaphragma* (Di) muscle containing only I and IIa MyHC is used as a control.

(b – c) Electrophoresis applied for the separation of MyHC of *Semitendinosus* (ST) muscle from Charolais fetuses of 60, 110, 180, 210 and 260 days post conception. Controls: adult *Cutaneus trunci* (CT) containing IIa and IIx MyHCs; adult ST containing I, IIa, IIx MyHC isoforms; adult *Diaphragma* (Di) containing I and IIa MyHCs

(b) Technique of Talmadge and Roy [61] allows the separation of developmental isoforms and not of fast adult MyHC isoforms.

(c) Technique of Picard *et al.* [40] allows the separation of adult MyHC isoforms but not of the developmental ones.

Figure 4. Labeling of muscle fibers from *Semitendinosus* muscle of Charolais fetuses at different stages of fetal life (same samples shown in **Figure 3b-c**) with an antibody specific to slow MyHC, and fetal MyHC according to Picard *et al.* [40].

Figure 5. Evolution of muscle fibers and their cross sectional areas as a function of the age of the animal.

(a) Post-natal evolution of the cross sectional area of fibers of *Semitendinosus* muscle from Blond d'Aquitaine young bulls. In red fiber sections stained with azorubine colorant.

(b) Modeling of the evolution with age of the proportions of the different type of fibers and their cross sectional areas [69].

Figure 6. Illustration of troponin T fast isoforms (fTnT) differentially expressed or not, between double muscled (DM) homozygote (DM), heterozygote (HDM), non-double muscled cattle (NDM) and between cattle selected (H) or not (L) on muscle growth potential [111]. * indicates exon 16, and spots without * come from exon 17.

Figure 7. Myosin heavy chains and proteomic biomarkers discriminate between rearing practices (adapted from Gagaoua *et al.* [31]) and meat color traits from PDO Maine-Anjou cows. The animals were clustered into three classes based on rearing practices[31]: Class 1 "C1= Hay", Class 1 "C2= Grass" and Class 3 "C3= Haylage". Among the biomarkers, myosin fibers IIA and IIX were able to separate the classes, especially C2 from C1 and C3. Accordingly, and due to high physical activity, the animals of "Grass" class have higher proportions of oxidative (IIA) at the expense of IIX fibers, a consequence of muscle plasticity. Furthermore, other beef tenderness protein biomarkers such as Superoxide dismutase (SOD1) and α-B-crystallin (CRYAB) were good discriminators of the rearinrn practices classes and beef color evaluated (a*: redness, b*: yellowness and C*: chroma). The grass class had lower amounts of MyHC-IIX whatever the technique used for their quantification, *i.e.,* a) by SDS-PAGE or b) by DOT-BLOT techniques.

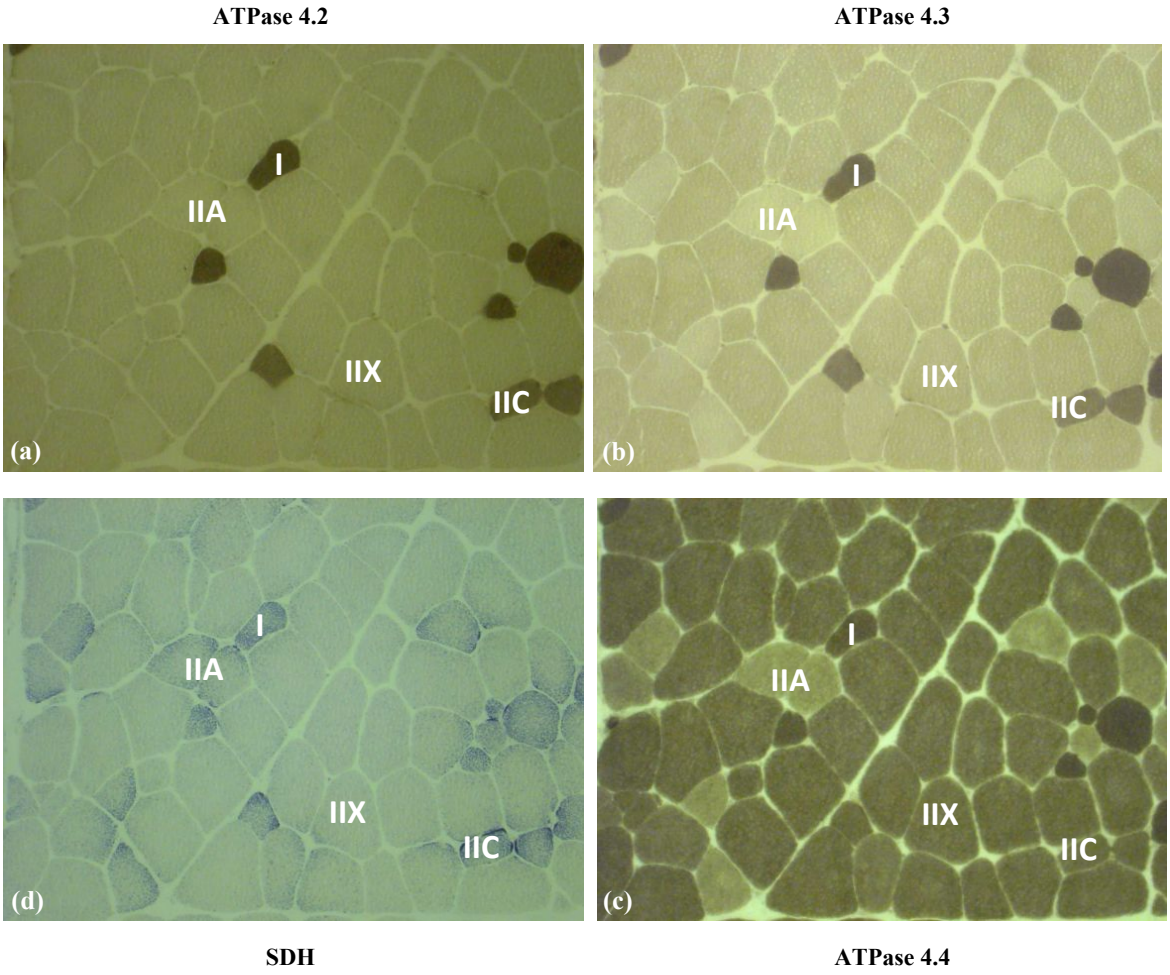
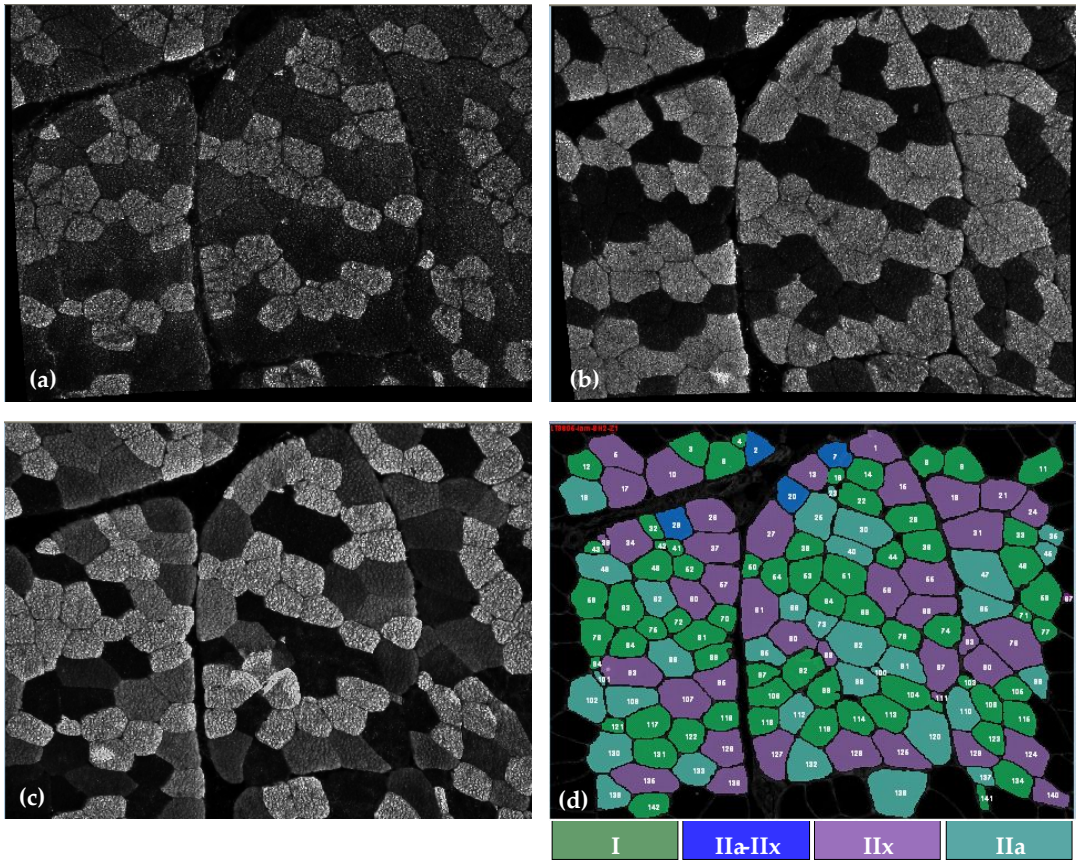


Figure 1.



1458 **Figure 2.**

1459

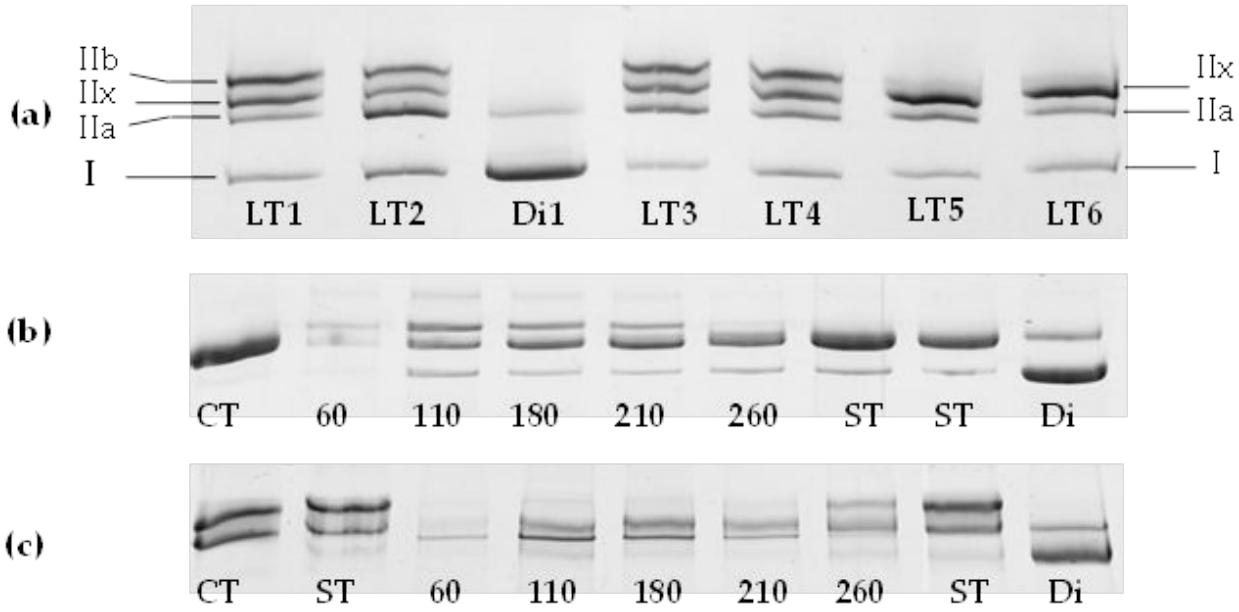

(a)
IIb
IIx
IIa
I
LT1
LT2
Di1
LT3
LT4
LT5
LT6
IIx
IIa
I
(b)
CT
60
110
180
210
260
ST
ST
Di
(c)
CT
ST
60
110
180
210
260
ST
Di

Figure 3.

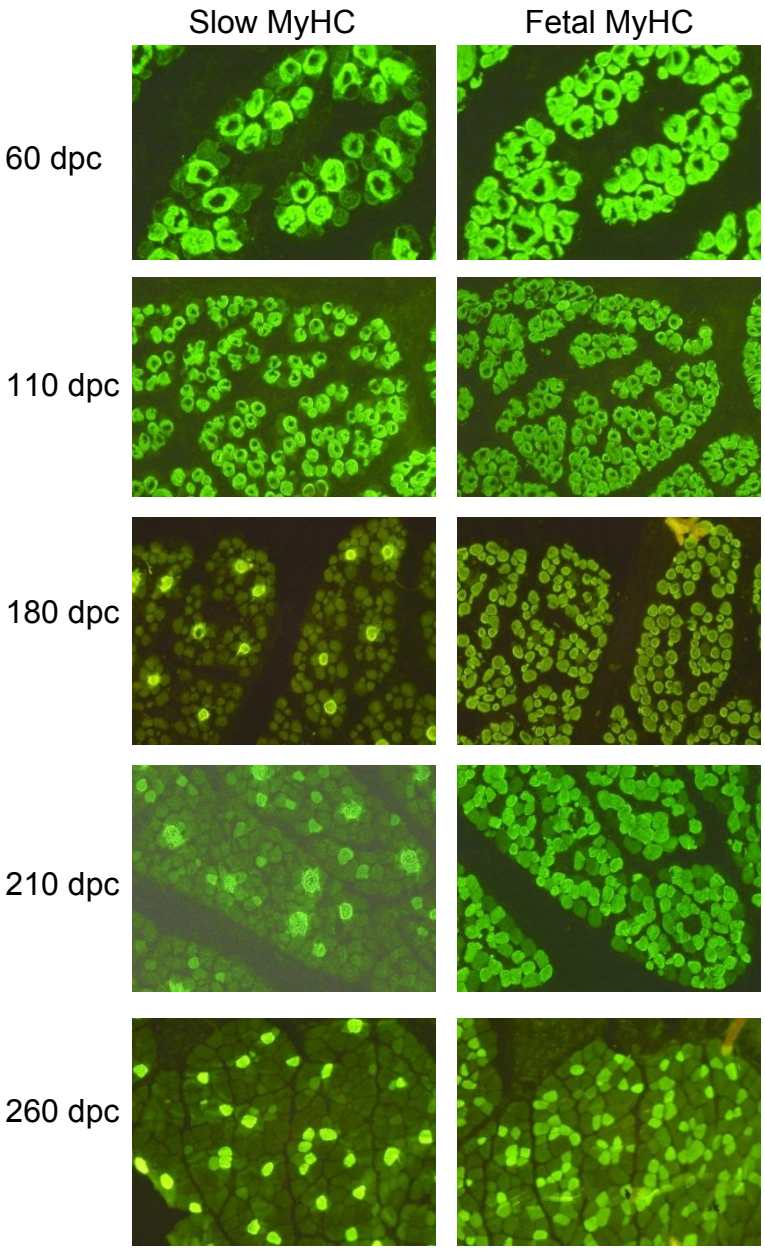


Figure 4.

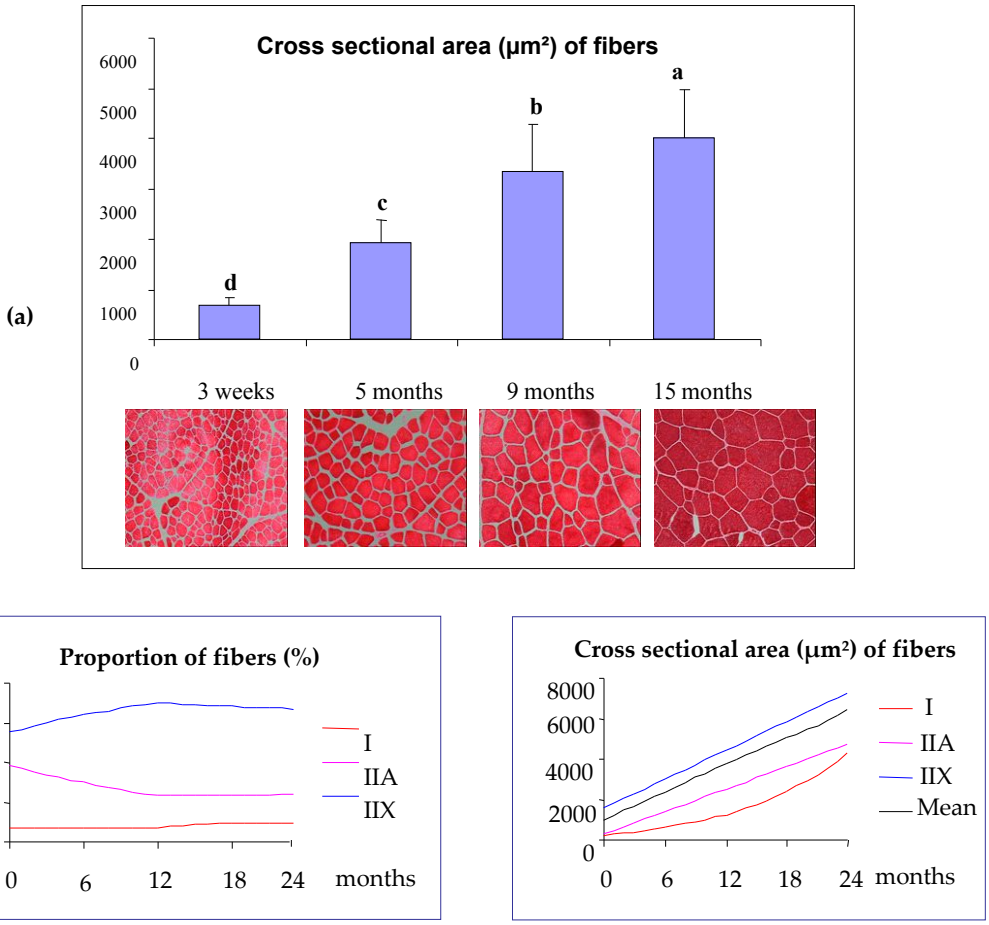

(a)
Cross sectional area (µm²) of fibers
6000
5000
4000
3000
2000
1000
0
d
c
b
a
3 weeks
5 months
9 months
15 months
(b)
Proportion of fibers (%)
80
60
40
20
0
0
6
12
18
24
months
I
IIA
IIX
Cross sectional area (µm²) of fibers
8000
6000
4000
2000
0
0
6
12
18
24
months
I
IIA
IIX
Mean

Figure 5.

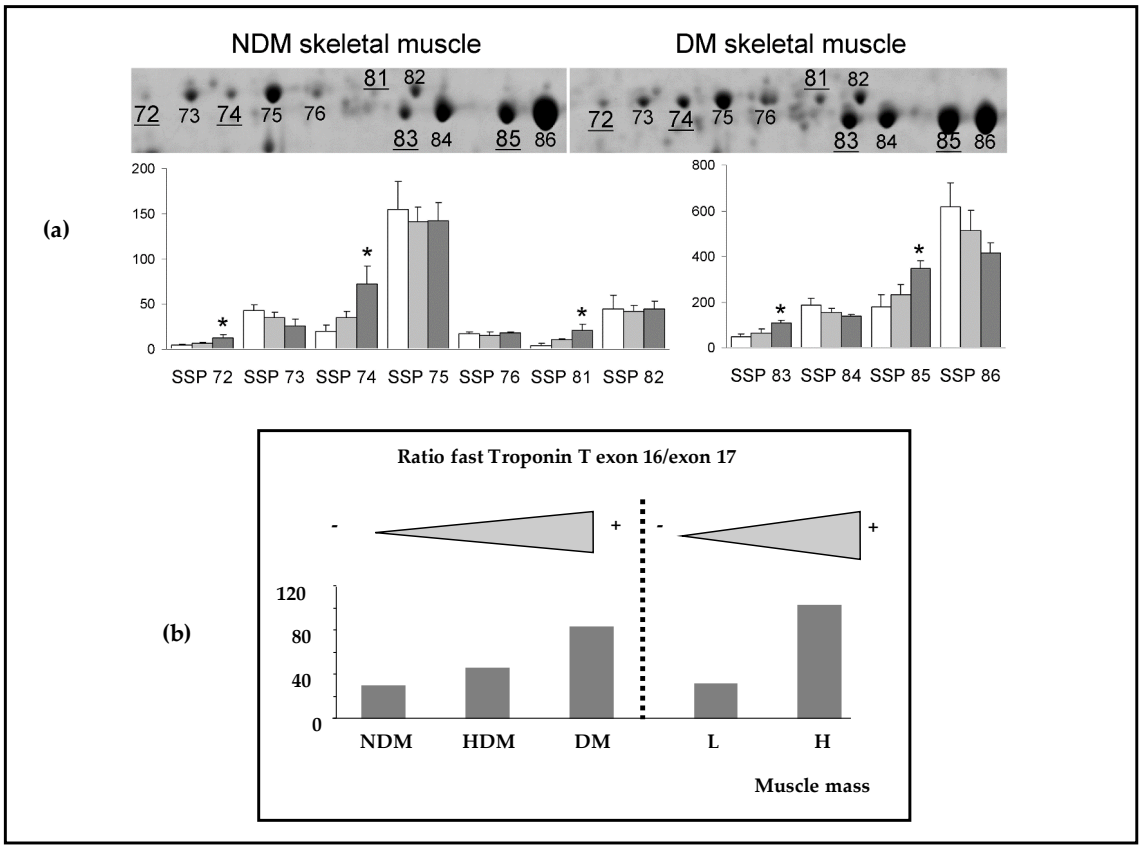

(a)
NDM skeletal muscle
DM skeletal muscle
72 73 74 75 76 81 82 83 84 85 86
200
150
100
50
0
800
600
400
200
0
SSP 72 SSP 73 SSP 74 SSP 75 SSP 76 SSP 81 SSP 82
SSP 83 SSP 84 SSP 85 SSP 86
(b)
Ratio fast Troponin T exon 16/exon 17
-
+
-
+
120
80
40
0
NDM
HDM
DM
L
H
Muscle mass

Figure 6.

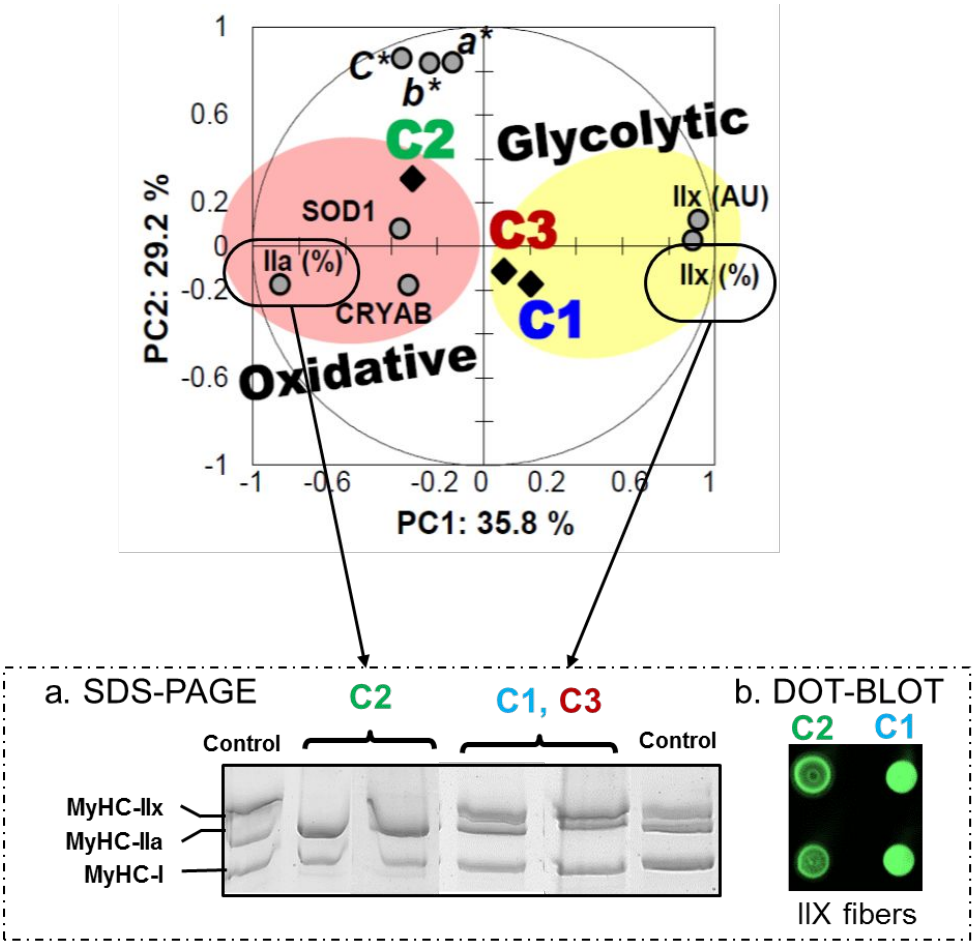

C*
a*
b*
C2
Glycolytic
SOD1
C3
IIx (AU)
IIa (%)
IIx (%)
CRYAB
C1
Oxidative
PC2: 29.2 %
PC1: 35.8 %
1
0.6
0.2
0
-0.2
-0.6
-1
-1
-0.6
-0.2
0
0.2
0.6
1
a. SDS-PAGE
C2
C1, C3
b. DOT-BLOT
C2
C1
Control
Control
MyHC-IIx
MyHC-IIa
MyHC-I
IIX fibers

Figure 7.

1514 **Table 1.** Main physiological and biochemical characteristics of muscle fiber types (adapted
1515 from [2, 5]).

Characteristics	I	IIA	IIX	IIB
Gene names	*MYH7*	*MYH2*	*MYH1*	*MYH4*
Full protein name	Myosin-7	Myosin-2	Myosin-1	Myosin-4
Uniprot ID	Q9BE39	Q9BE41	Q9BE40	E1BP87
Contraction speed	+	+++	++++	+++++
ATPase activity	+	+++	++++	+++++
SDH activity	+++++	++++	++	+
Contraction threshold	+	+++	++++	+++++
Contraction time per day	+++++	++++	+++	+
Fatigue resistance	+++++	++++	++	+
Oxidative metabolism	+++++	++++	++	+
Glycolytic metabolism	+	++++	++++	+++++
Phosphocreatine	+	+++++	+++++	+++++
Glycogen	+	+++	+++	++++
Triglycerides	+++++	+++	+	+
Phospholipids	+++++	++++	+++	+
Vascularization	+++++	+++	+, ++	+
Myoglobin	+++++	++++	++	+
Mitochondria	++++	+++	++	+
Buffering capacity	+	++++	+++++	+++++
Z line width	+++++	+++	+++	+
Cross sectional area	+	++	++++	+++++
Red color	+++++	+++	++	+

+, very low; ++, low; +++, medium; ++++, high; +++++, very high.

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Muscles fibers from fetal life to slaughter and relation with meat quality