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Skeletal muscle proteomics in livestock production

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Abstract

Proteomics allows studying large numbers of proteins, including their post-translational modifications. Proteomics has been, and still are, used in numerous studies on skeletal muscle. In this article, we focus on its use in the study of livestock muscle development and meat quality. Changes in protein profiles during myogenesis are described in cattle, pigs and fowl using comparative analyses across different ontogenetic stages. This approach allows a better understanding of the key stages of myogenesis and helps identifying processes that are similar or divergent between species. Genetic variability of muscle properties analysed by the study of hypertrophied cattle and sheep are discussed. Biological markers of meat quality, particularly tenderness in cattle, pigs and fowl are presented, including protein modifications during meat ageing in cattle, protein markers of PSE meat in turkeys and of post-mortem muscle metabolism in pigs. Finally, we discuss the interest of proteomics as a tool to understand better biochemical mechanisms underlying the effects of stress during the pre-slaughter period on meat quality traits. In conclusion, the study of proteomics in skeletal muscles allows generating large amounts of scientific knowledge that helps to improve our understanding of myogenesis and muscle growth and to control better meat quality.

Keywords: *proteomics; muscle; meat quality; myogenesis*

INTRODUCTION

The term Proteome was used for the first time in 1994 by Marc Wilkins in Italy. Since then, the technique has evolved with increasing numbers of identified proteins and sequenced genomes. Proteomics allows the study of quantitative and qualitative variations in hundreds of proteins. It is complementary to transcriptomics as protein abundance is not the simple reflection of mRNA expression [1–3].

The development of proteomics was made possible by technical progress made in electrophoretic and chromatographic separations and subsequent mass spectrometer analysis. Proteomics can generate large data sets which can be analysed with high-throughput bioinformatic tools allowing identification of molecule interactions and analysis of molecular pathways, i.e. chains of chemical or physical interactions in which the product of one reaction becomes the reactant of the other. As the amount of information in pathway databases grows, efforts to rationalise this information increase and better definitions of molecular pathways emerge [4]. Proteome mapping has various constraints. For example, results depend on the pH gradient chosen at the start of the

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experiment. Thus, in a previous experiment using proteomics, separation of proteins from bovine skeletal muscle using a 4–7 pH gradient in the first dimension allowed the detection of roughly 500 reproducible protein spots [5]. Among them, 129 were identified and mapped. This first map was completed using a 7–11 basic pH gradient, allowing the mapping of 60 more proteins [6]. By combining these two methods, it was possible to produce, with a good resolution and reproducibility, maps of bovine muscle proteins with a pI between 4 and 11 pH units.

After translation, most proteins are covalently modified by post-translational modifications such as phosphorylation, glycosylation, methylation, nitration, sulfatation, fatty acid addition or ubiquitination, and these different forms of proteins can be evidenced by proteomics. For example, Scheler *et al.* [7] were able to detect over 30 isoforms of Heat Shock Protein (HSP) 27 kDa in cardiac muscle, which may be explained by different degrees of phosphorylation. About 20% of the ~200 amino acids constituting the HSP27 molecule are potential phosphorylation sites [8].

Proteomics may also be used to study upstream regulation of proteins. For example, Bouley *et al.* [9] studied the origin of different isoforms of Troponin T (TnT). TnT molecules are encoded by three genes characteristic of cardiac, slow and fast-twitch skeletal muscles, respectively. Using two-dimensional electrophoresis (2DE), Bouley *et al.* [5] revealed 17 TnT isoforms in bovine ST muscle, 6 belonging to the slow type and 11 to the fast type. Using MS/MS analysis they showed that these different fast isoforms originated partly from exclusive alternative splicing of exon 16 and 17 in the mRNA molecules [9]. Such analyses of post-translational modifications are often essential for increasing our understanding of biological pathways.

Over the last years, proteomic tools were used for various applications in muscle and meat science (for reviews [10, 11]). In this review, we focus on muscle studies in various livestock species conducted to understand better biochemical mechanisms underlying muscle mass and meat quality.

MUSCLE ONTOGENESIS

Meat is an important end product of livestock production. Muscle growth and intrinsic properties of the muscle determine at least in part the quantity and quality of the meat produced. Proteomics presents an interesting tool to increase our knowledge of muscle properties and how they develop during myogenesis.

Many proteomic studies of myogenesis used *in vitro* models of C2C12 lines. For example, Tannu *et al.* [12] identified 653 proteins in myoblasts and 558 proteins in myotubes; 106 of these showed different abundance depending on the cell type. Proteins with a major role in differentiation were identified as mitogen activated protein kinase (MAPK), phosphorylated alpha 1 catalytic subunit isoform 1 (pAkt), protein kinase B (PKB), the kinase p38, phosphorylated extracellular regulated kinase (pERK), serine/threonine protein kinase (Akt2/PKB), IGF1 receptor and caspase 3. The analysis of the phosphoproteome showed that myoblast differentiation requires activity of numerous kinase proteins [13]. For example, proteins such as MAPK, Akt, c-Jun N-Terminal Protein Kinase 1 (JNK1) or cyclin dependant kinase 5 (CDK5) are essential for myogenesis. Phosphoproteins involved in neuronal differentiation such as stathmin and intracellular serine protease (LANP) also appeared to be important for myogenesis. Chan X'avia *et al.* [14] showed that moesin, fibronectin and pro-collagen migrate in the extracellular matrix (ECM) during differentiation and that other proteins such as serpin, pigment epithelium-derived factor (PEDF), annexin1 and galectin1 have an important role in cellular migration. These *in vitro* studies have been recently completed by *in vivo* analyses conducted in livestock animals such as cattle, pigs and chickens throughout myogenesis. In these species, the development of muscle fibres has been well described using histochemical and biochemical approaches [15]. To increase our understanding of mechanisms controlling myogenesis, changes of muscle proteome during foetal life were investigated at physiologically comparable stages in these three species (Figure 1). Proteomic analyses were carried out using 2DE as described by Bouley *et al.* [5] and mass spectrometry. The developmental stages studied correspond to: (i) proliferation of the first generation of myoblasts, (ii) proliferation of the second generation of myoblasts and differentiation of the first generation, (iii) end of proliferation of myoblasts and differentiation of myotubes (Total number of fibres (TNF) is fixed), (iv) contractile and metabolic maturation of myotubes and fibres.

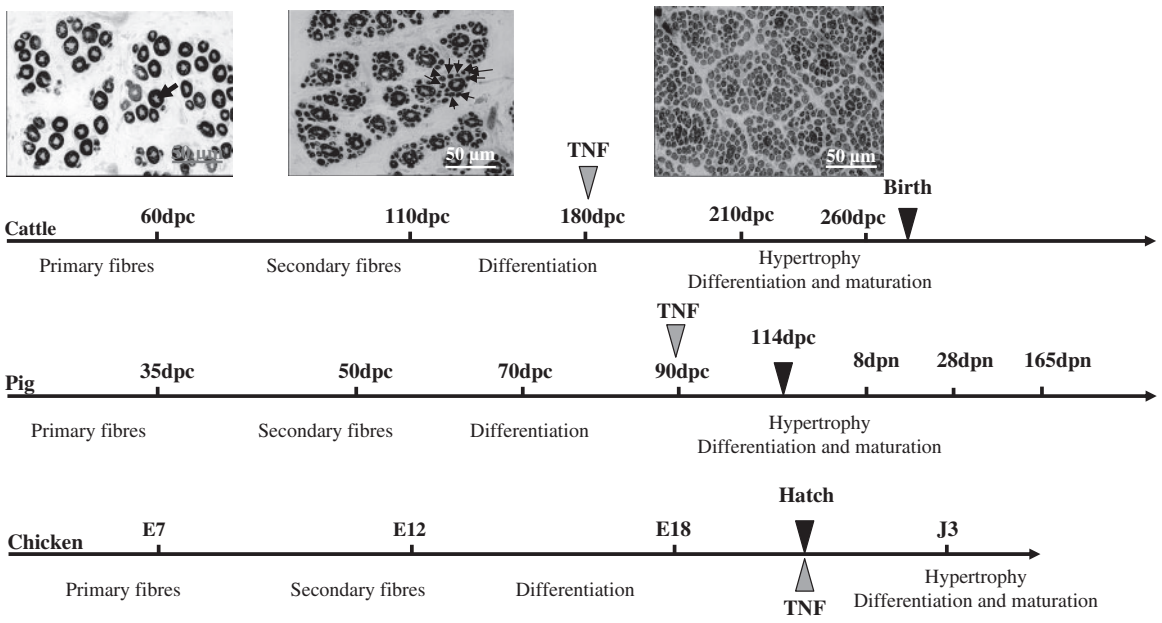

Figure 1: Comparable physiological key stages of myogenesis in different species. Cattle: 60 days post-conception (dpc; proliferation of the first generation of myoblasts), 110 dpc (differentiation of the first generation of myotubes and proliferation of the second generation of myoblasts), 180 dpc (end of differentiation of the second generation of myotubes; total number of fibres (TNF) is established), 210 and 260 dpc (contractile and metabolic maturation of fibres) [16, 17]. Pigs: TNF is established by 90 dpc, whereas maturation in terms of contractile and metabolic properties occurs essentially from the end of gestation up to the first month after birth, occurring at 113 dpc [18]. Pig myogenesis is clearly a biphasic phenomenon with primary myotubes forming from 35 to 55 dpc, followed by secondary myotubes forming around each primary myotube between 50 and 90 dpc. The secondary/primary myotube ratio varies between 20 and 30 depending on the muscle and breed [19]. Around birth, some secondary myotubes in the close vicinity of each slow primary myotube mature to slow-twitch type I fibres giving rise to the typical rosette distribution consisting in islets of type I fibres surrounded by fast-twitch type II fibres in pig skeletal muscles [20]. Chickens: stages 7 and 12 d *in ovo* correspond to the formation of the first and second generation of myoblasts, stage 18 d *in ovo* (i.e. about 3 days before hatching) to the differentiation of muscle fibres, and 3 days post-hatch to the contractile and metabolic maturation of fibres [21, 22].

Myogenesis in cattle

Proteomic analysis of cattle *semitendinosus* muscle revealed that 496 spots were common to the five stages studied (Figure 1) [16, 17]. Overall, the abundance of 245 proteins changed during the nine months of gestation (Figure 2A and B). Principal Component Analysis and Hierarchical Clustering identified up to eight different profiles of protein kinetics corresponding to several biological functions.

The most abundant proteins during the first stage (60 dpc) (clusters 6, 7, 8 in Figure 2A) corresponded mainly to embryonic tissue development, and regulation of cell growth and organisation. For example, Rho GDP dissociation inhibitor (RhoGDI) alpha protein, highly expressed at this stage, is involved in cytoskeleton reorganisation during cell proliferation. The second stage, 110 dpc, is the most complex as it corresponds to many Gene Ontology (GO) terms relative to proliferation, differentiation and fusion, amongst others. This is consistent with the fact that different populations of cells are present at this stage, such as differentiating primary myotubes and proliferating secondary myoblasts [15]. For example, expression of Galectin 1 is highest at this stage, in accordance with its involvement in myoblast fusion [23].

Proteins involved in apoptosis are also abundant at 60 and 110 dpc (cluster 7, Figure2A), suggesting that the proliferation–apoptosis balance may play a role in the determination of TNF [16]. Among them, many proteins, including an ATP-dependent DNA helicase (RuvBLike2), heterogeneous nuclear ribonucleoprotein K (hnRNPK), tryptophanyl-tRNA synthetase (WARS), mitochondrial chloride intracellular channel 4 (CLIC4) and DJ-1 protein have been described to play a role in apoptosis. Another protein

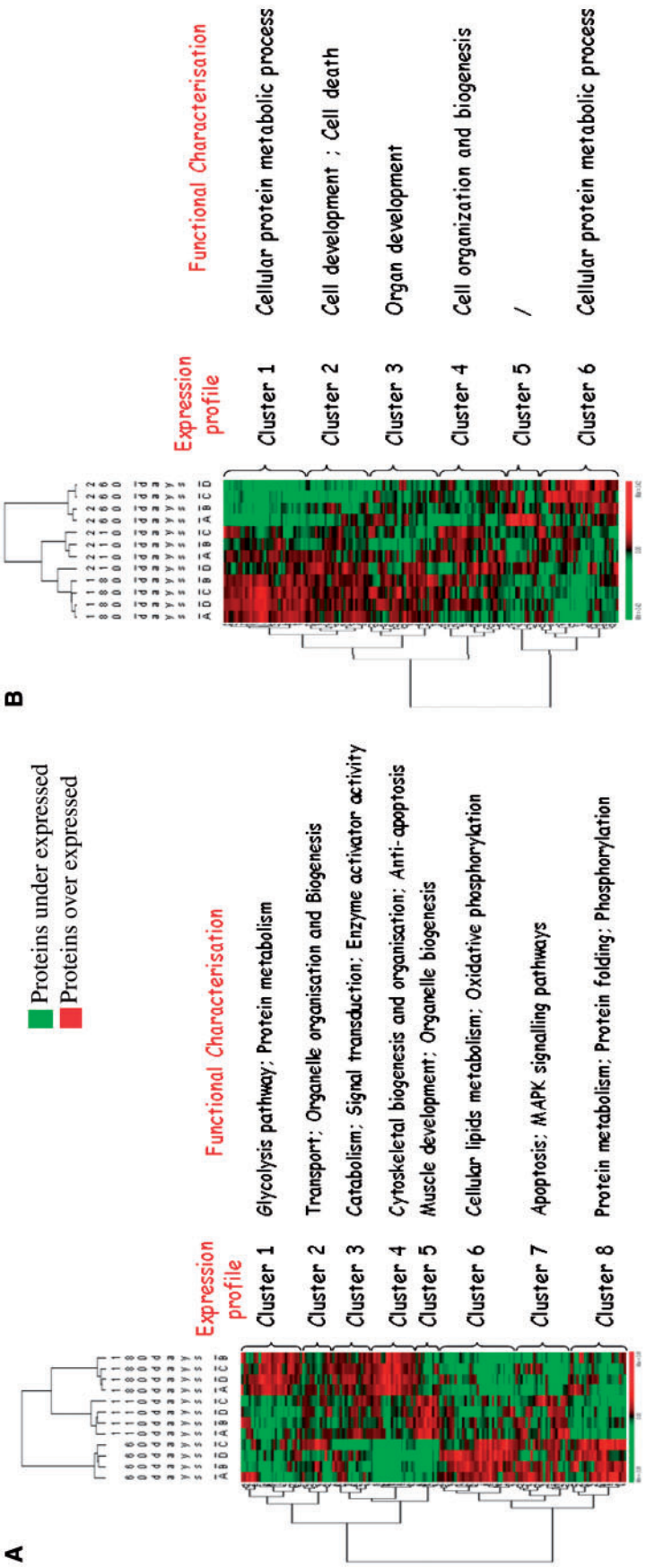

Figure 2: Hierarchical classification and characterization of (**A**) proteins present at the three early stages of cattle foetal life (60, 110 and 180 dpc) and (**B**) proteins present at the three late stages of cattle foetal life (180, 210 and 260 dpc), according to Chaze *et al*. [16, 17], respectively. Individual samples are identified by columns: stages 60 to 180 (**A**), 180 to 260 days post conception (**B**).

with anti-apoptotic properties, HSP27, showed high abundance during the first two-thirds of cattle foetal life [16]. It is possible that these proteins play a role in the determination of TNF acquired at 180dpc for *Bos taurus* [15]. TNF is of particular interest in meat production since it is one of the most important parameters that control muscle mass [16]. A major component of high density lipoprotein cholesterol, apolipoprotein A1 (ApoA1**),** also showed high levels at this stage [16].

Stage 180 dpc corresponds to a transition between the formation of myofibres (TNF is fixed from this stage onwards [15]) and their maturation fate, which is supported by proteomic data. Several changes indicate a reduction in cell proliferation. Thus, stathmin, which has an important role in cell cycle regulation showed decreased expression from 180 dpc onwards. Septin proteins (septin 2 and 11 isoforms) involved in cytoskeletal organisation, scaffolding and division plane had a stable expression up to 180 dpc, confirming intense cell division until this stage, and declined thereafter. In parallel, annexin A1, which has an anti-proliferative function via the activation of ERK pathways, showed increased abundance from 180 dcp onwards [16, 17]. Increased maturation is reflected by significant changes in the profiles of protein isoforms belonging to metabolic and contractile pathways (cluster 1, Figure 2B) [17]. Thus, proteins of the aldehyde dehydrogenase family, involved in the metabolism of amino acids, carbohydrates and lipids, dihydrolipoyl dehydrogenase, involved in pyruvate metabolism, as well as enolase, lactate dehydrogenase (LDH) and isocitrate dehydrogenase (ICDH), all enzymes involved in carbohydrate metabolism, showed increased abundance from 180 dpc onwards [17]. Similar results were found for proteins of the contractile apparatus. Abundance of different isoforms of myosin light chains (MyLC) 1 and 2, alpha-cardiac actin, fast TnT, myosin-binding protein (MyBP) and adult forms of MyHC increased during the later stages of foetal life [17].

Pig myogenesis

The study of muscle proteome during myogenesis (Figure 1) revealed a total of 870 spots detected among the developmental stages. Principal Component Analysis on centred and normalised data showed that 2DE gels could be grouped according to the stage of development with a gradual shift from 45 dpc to 165 d postnatal, denoting that the proteomic approach was physiologically relevant [24]. Among the 870 spots, 295 could be classified into 7 clusters based on the longitudinal profile of their expression. So far, 20% of the 295 spots could be identified using MALDI-TOF mass spectrometry probably due to the low intensity of many spots. Among the identified proteins, several were highly expressed during the early foetal period, such as transferrin, fetuin, angiotensinogen, transthyretin, atrial/embryonic MyLC1 and calponin 3. Other proteins peaked during the perinatal period such as HSP27, voltage-dependent anion channel 1 and mitochondrial F1 ATPase, whereas a large number of the identified proteins, about 60%, increased postnatally and mostly corresponded to glycolytic enzymes, creatine kinase M, different isoforms of MyLC, fast TnT and myoglobin, which corresponds to the postnatal maturation of contractile and metabolic properties of pig skeletal muscle [18]. Because of the high prolificacy in the pig species, it is common that some piglets suffer of intrauterine growth restriction. It is well documented that low birth weight piglets have a lower TNF than heavier littermates [25], which permanently reduces their postnatal muscle growth potential [26–28]. Interestingly, the study on myogenesis found that transferrin, a protein involved in iron transport, was more expressed ($P < 0.01$) in *longissimus* muscle of high than medium and low weight foetuses at 70 dpc (Figure 3), suggesting that transferrin could influence foetal growth, myogenesis and TNF. Another study comparing the proteome of the *gastrocnemius* muscle between low and normal birth weight newborn piglets identified 12 differentially expressed proteins [29]. These results show that expression of proteins involved in protein synthesis, antioxidant function, catecholamine degradation and fat metabolism was reduced in intrauterine growth restricted piglets, whereas expression of mitochondrial F1-ATPase and proteasome was increased. These changes may at least partly explain the restricted intrauterine growth and lower survival at birth of these piglets.

Other studies compared the proteomes between red and white pig skeletal muscles [30], genotypes, rearing conditions and gender [31], or analyzed changes in muscle proteome during compensatory growth in the postnatal period [32]. These studies showed that slow MyLC, myoglobin and HSP20 were higher in red than white muscles, whereas the opposite was observed for HSP27.

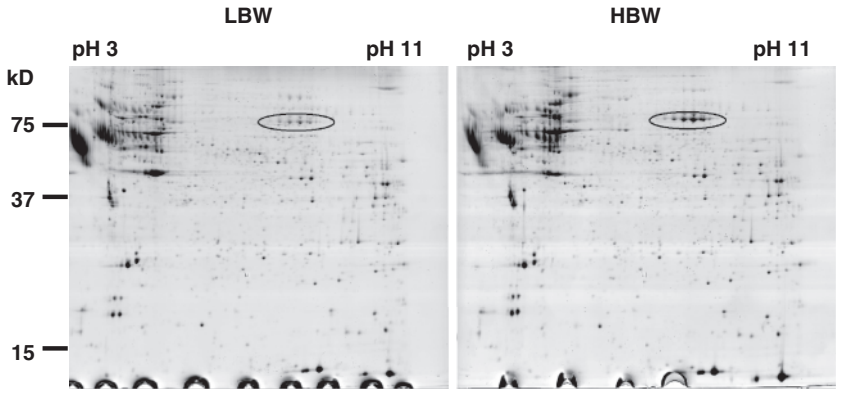

Figure 3: Representative Coomassie blue G250 stained 2DE gels of *longissimus* muscle proteomes between low (LBW, 170 g) and high (HBW, 280 g) body weight Large-White pig foetuses at 70 dpc. Proteins (300 μg) were loaded and separated on a nonlinear 3–11 pH gradient strip in the first dimension and a 12.5% acrylamide SDS gel in the second dimension. Tansferrin isoforms surrounded by an ellipse are more highly expressed in HBW foetuses.

Compensatory growth induced a decrease in spot intensity of HSP70, HSP27, enolase 3 and glycerol-3-phosphate dehydrogenase (GPDH), denoting a decrease in glycolytic metabolism compared to continuously *ad libitum* fed pigs.

Chicken myogenesis

In chicken thigh muscles, the dynamic proteomic approach revealed that 531 spots were present at each of the stages included in the analysis (i.e., 7, 12, and 18 days *in ovo*, and 3 days post-hatch) (Figure 1). Hierarchical clustering analysis [33] showed that the expression of 168 of them was affected by developmental stage (Figure 4). The proteins identified as differentially expressed between stages could be classified into 9 clusters based on their expression profile: two clusters comprised proteins showing increasing abundance during myogenesis and three clusters contained proteins showing decreasing abundance during myogenesis. The four other clusters contained proteins transiently expressed during the *in ovo* development.

Approximately 100 out of the 168 proteins could be identified using MALDI-TOF mass spectrometry. Among the proteins showing progressively increasing expression during early muscle development, several were involved in energy metabolism (α and β enolase, creatine kinase, NADH dehydrogenase, cytochrome c reductase, succinyl CoA ligase), lipid transport (apolipoprotein B, fatty acid-binding protein 3) or calcium signalling (sarcalumenin). Regarding β enolase and creatine kinase, their expression greatly increased during postnatal muscle growth, whereas that of α enolase remained stable [34]. Other proteins were related to the contractile apparatus such as MyLC isoforms and proteins interacting with contractile proteins, such as an isoform of cofilin-2, which inhibits the polymerisation of monomeric G-actin, and T-complex polypeptide-1 (TCP1, subunit theta) which interacts with tubulin. Prohibitin, known to have an antiproliferative activity, gradually increased until day 18 *in ovo*, when cell proliferation slows down and differentiation starts.

Some proteins were expressed during embryogenesis but not after hatch. These proteins included alpha-fetoprotein, regucalcin and proteasome 26S subunit, all predominantly expressed during the early stages of myogenesis (day 7 *in ovo*). Alpha-fetoprotein is a major plasma protein produced by the yolk bag and the liver during foetal life. The protein is thought to be a member of the albumin family and to play a role in the regulation of growth and immune function, and has possibly an anti-apoptotic action [35, 36]. Regucalcin regulates intracellular Ca^{2+} homeostasis by regulating Ca^{2+}-transport systems in liver and renal cells [37]. Hepatic regucalcin mRNA levels were found to be increased during foetal development and liver regeneration in rats, and influenced by maternal calcium intake [38, 39]. Proteasome contains large protein complexes that degrade unneeded or damaged proteins through proteolysis [40] and were also found to be expressed during foetal development in muscles of the Landrace, but not the Tongcheng pig breed [41]. The expressions of retinol-binding proteins, involved in cell differentiation, remained stable during *in ovo* development but decreased markedly after hatch. The same pattern of expression was also observed

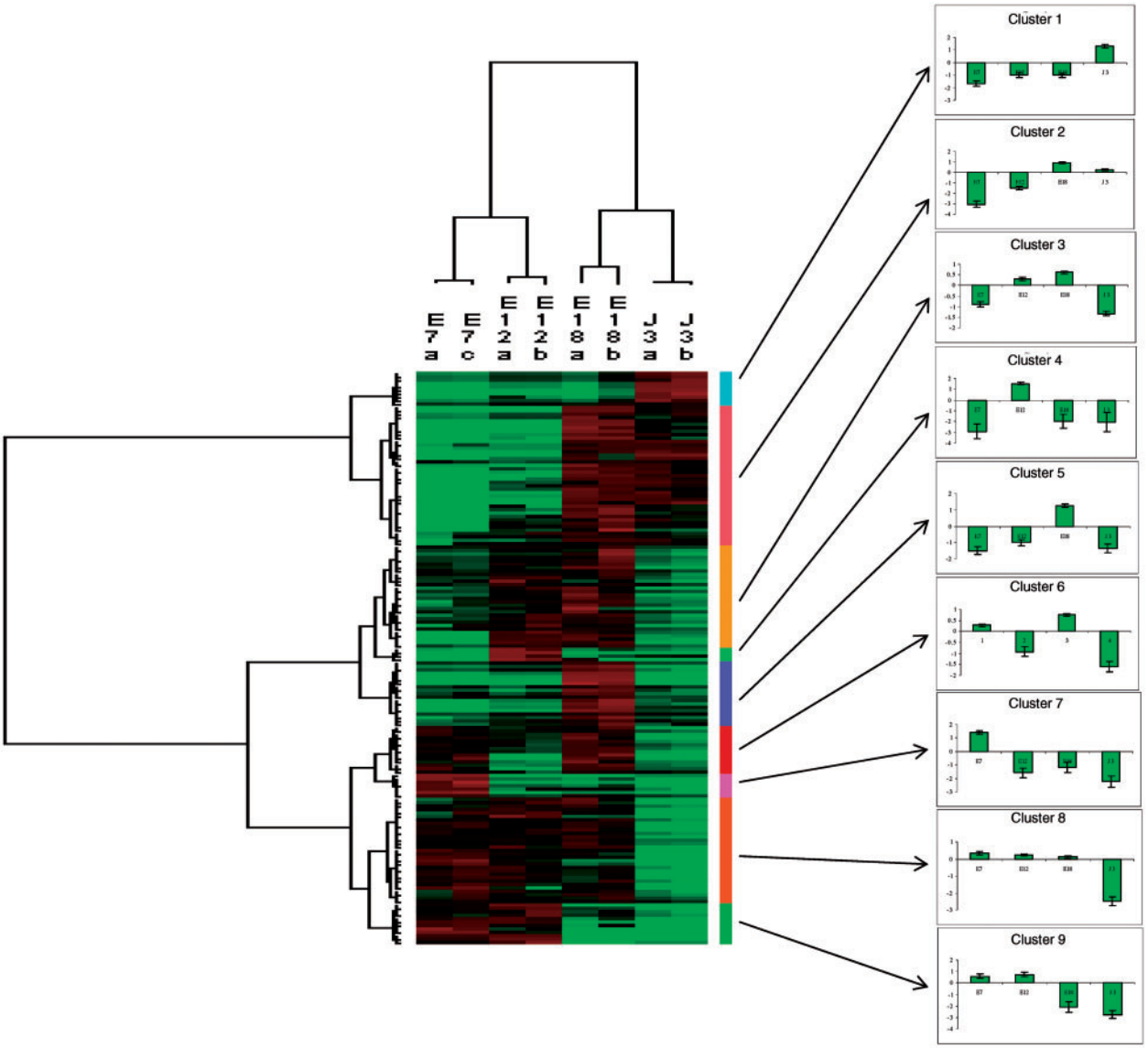

Figure 4: Hierarchical clustering classification of proteins identified by 2DE gels in chicken thigh muscles during development. The stages E7, EI2 and EI8 and J3 correspond to day 7, I2 and I8 *in ovo* and day 3 post-hatch, respectively. The proteins are classified into 9 clusters of which patterns of expression are described on the right side of the figure.

for the eukaryotic translation initiation factor 4E (eIF4E) which is involved in several cellular processes including enhanced translational efficiency, splicing, mRNA stability and RNA nuclear export and constitutes a rate-limiting component of protein synthesis. By contrast, the eukaryotic translation initiation factor 3 (eIF3I) which interacts with transforming growth factor beta (TGF-beta) was overexpressed at stages 7 and 18 d *inovo* when the formation of primary myoblasts and the differentiation of muscle fibres begin, respectively. Several other proteins also showed transiently increased expression during muscle early development. At 18 days *in ovo*, several actin-related proteins were transiently overexpressed, such as actin-related protein 3, F-actin capping protein alpha 1 and 2, gelsolin and vimentin. ECM-related proteins like leprecan and thioredoxin also showed increased abundance at this stage, as well as several HSP such as HSP70, HSP70 protein 9 precursor (GRP75), and activator of HSP90 ATPase protein 1 (AHA1). HSP27 was not classified in the same cluster as the other HSP, but its expression was also maximal at 18 days *in ovo*. Finally, peaked expression was found at 18 days *inovo* for annexins 5 and 6, which

are membrane-binding proteins [42], and, similarly to cattle, ApoA1. The ApoA1 expression level was reported to gradually decline during postnatal muscle development in chickens [34, 43]. As also observed in cattle and pigs, the abundance of many proteins related to the contractile apparatus increased at this stage, likely in relation to the intensification of cell differentiation and maturation, concomitantly with a slowing down of cell proliferation.

Various enzymes showed progressively decreasing abundance *in ovo*. Some of them are known to interact with the cytoskeleton, such as stathmin, isoforms of TCP1 other than the theta subunit mentioned above (subunits beta, eta, epsilon), cofilin-2 (another spot than the one mentioned above), profiling-2, which like cofilin-2 inhibits the polymerisation of monomeric G-actin. Other enzymes are known to be involved in the ECM protein formation, such as thioredoxin, prolyl 4-hydroxylase alpha polypeptide II (P4HA2) and protein disulfide isomerase associated 3 (PDIA3), or in energy metabolism, such as B-creatine kinase and pyruvate dehydrogenase.

Comparative biology

Comparisons between the three species (cattle, pigs and chickens) show that temporal changes in expression of some proteins were similar (Figure 5). For example, proteins such as alpha-fetoprotein, albumin, fetuin and transferrin were more abundant during the early stages, and decreased thereafter. This indicates that these proteins likely play a major role in early myogenesis in the different species, including mammals and birds. Another example is α enolase showing decreasing expression during foetal or late *in ovo* life, whereas expression of β enolase increased. The opposite changes in α and β enolase isoforms were confirmed by western blot using specific antibodies in bovine muscle (Figure 5). Subsequent immunohistochemical studies using a specific α enolase antibody (Acris Enol BP087, 1/10) showed that the presence of α enolase was restricted to slow fibres arising from the first generation of myoblasts (data not shown). Further analysis is needed to determine if α enolase can be a good marker of primary fibres in the different species.

The three species showed a peak of HSP27 expression at a stage corresponding to sustained fibre differentiation or maturation (Figure 5), i.e. at 180–210 dpc in cattle, 18 days *in ovo* in chickens and during the perinatal period in pigs, characterised by intense contractile and metabolic maturation in this species. This may suggest that HSP27 plays a major role in the transition between proliferation of myoblasts and the differentiation and/or maturation of myofibres in the different species, possibly through its anti-apoptotic actions or through its stabilising

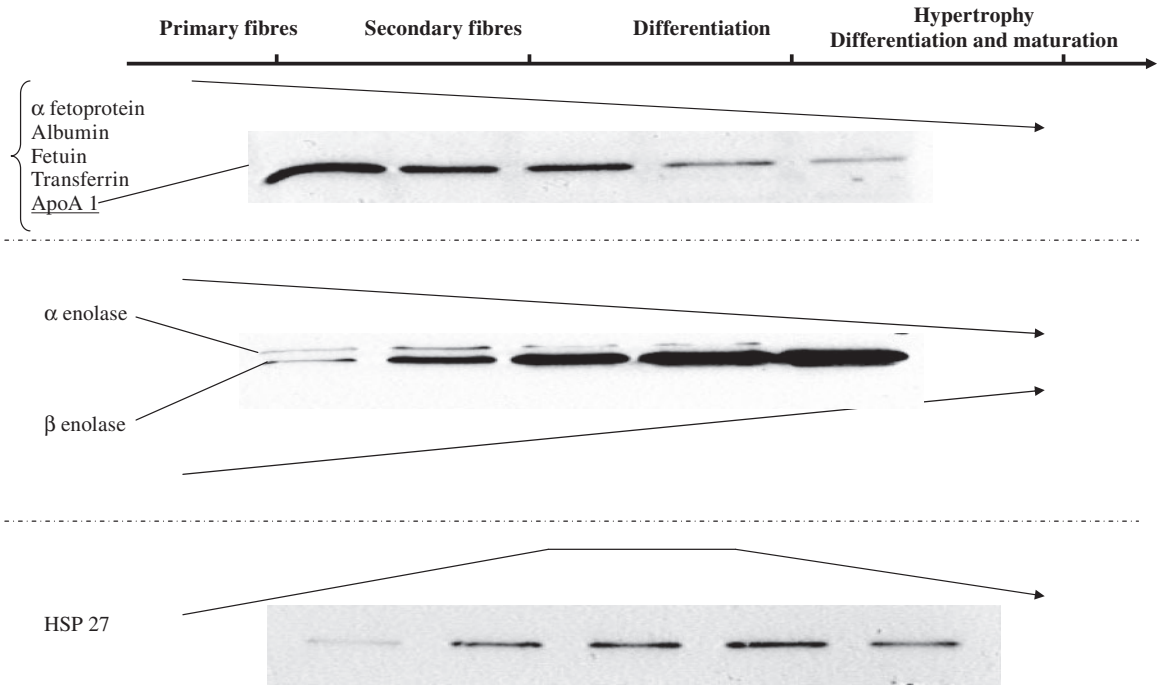

Figure 5: Common protein changes during myogenesis in cattle, pig and chicken as revealed by proteomic analysis.

action on myofibrils [44]. Other hypotheses are also possible. For example, one study found that the amount of extracted HSP27 was correlated with the amounts of extracted MyLC [31], indicating that HSP27 could simply reflect the amount of myofibrils in place.

The comparative approach also allowed the detection of proteins not known to have any role in muscle physiology. Thus, we observed high abundance of ApoA1 during the early stages of myogenesis and a decrease during the last stages both in bovine and chicken muscle. This progressive decrease was confirmed using western blot and immunohistochemical analyses in bovine muscle (Figure 5, ApoA1 polyclonal antibody from D. Bauchart; INRA Clermont-Ferrand/Theix). ApoA1 is known for its involvement in cholesterol transport and lipid metabolism [45]. Its expression in muscle fibres was not described before and its exact role in myogenesis remains to be elucidated. In all species, the proteomic analysis showed that changes occurring at the end of gestation or during the perinatal period mostly corresponded to the maturation of contractile and metabolic properties of skeletal muscle. Further analyses will help identifying more proteins and related GO terms to understand myogenesis in different farm animal species and identify common as well as divergent mechanisms between species.

GENETIC VARIABILITY: MUSCLE HYPERTROPHY

Genetic background has a strong impact on muscle growth. This is well illustrated in cattle by the double muscling phenotype based on mutations or deletions within the myostatin gene which lead to muscle hypertrophy. Increased muscle growth rate can also be obtained by conventional quantitative genetic selection. In this case, it is expected that the increased muscle growth rate is related to several, rather than to a single gene. We have investigated the effects of increased muscle growth on muscle proteome in these two situations. In one study, homozygous double-muscled Belgian Blue bulls (DM), heterozygous double-muscled (HDM) and homozygous non-double-muscled (N) animals were compared [9]. The double-muscling was related to a 11 base-pair deletion in the myostatin gene. In cattle, the loss of myostatin function induces an increase in skeletal muscle mass essentially through an increase of TNF [46]. In the other study, two lines of Charolais bulls differing in muscle growth rate, i.e. high (H) or low (L), were compared. In both studies, differential proteomic analysis of the mixed fast glycolytic semitendinosus muscle was performed using 2DE gels (4–7 pH gradient in the first dimension) followed by MALDI-TOF mass spectrometry analysis of differentially expressed spots [5].

Among the proteins showing different abundance in Belgian Blue DM compared to HDM and N muscles, two were involved in metabolic pathways: phosphoglucomutase (PGM) was more abundant and heart fatty acid-binding protein (H-FABP) was less abundant in DM muscles. Two muscles-specific proteins, sarcosin and sarcoplasmic reticulum 53-kDa glycoprotein, showed increased, and a HSP-like protein, p20, showed decreased abundance in DM muscles. However, most of the proteins which were influenced by the DM condition were related to the contractile apparatus. Those showing increased abundance in DM muscle, including myosin-binding protein H, MyLC2 and MyLC3, are more specifically expressed in fast-twitch glycolytic muscle fibres. In contrast, MyLC2s, MyLC1sa, MyLC1sb and slow TnT, proteins known to be more abundant in slow-twitch oxidative muscle fibres, showed lower abundance in DM muscle. Interestingly, mRNA splicing of fast TnT at the mutually exclusive exons 16 and 17 was influenced by the loss of myostatin function. Thus, the fTnT molecule with exon 16 was predominantly expressed in DM muscles (Figure 6A and B). Therefore, the fTnT exon 16 / fTnT exon 17 ratio was found to be a good marker of muscle mass development [9]. HDM muscles exhibited generally intermediate levels of these proteins.

Results obtained on the conventionally selected young Charolais bulls were in accordance with those obtained on the Belgian Blue bulls. Charolais bulls with increased muscle growth rate had increased abundance of various isoforms of MyLC specific of fast-twitch glycolytic fibres, and decreased abundance of MyLC isoforms more specific of oxidative fibres [47, 48]. Similarly to the DM Belgian Blue bulls, increased muscle growth was associated with increased abundance of fTnT containing exon 16 and decreased abundance of sTnT and fTnT containing exon 17 [47] (Figure 6B). In accordance with the Belgian Blue bulls, Charolais bulls with increased muscle growth rate had decreased enzyme activity of citrate cynthase (CS) and cytochrome-c oxydase

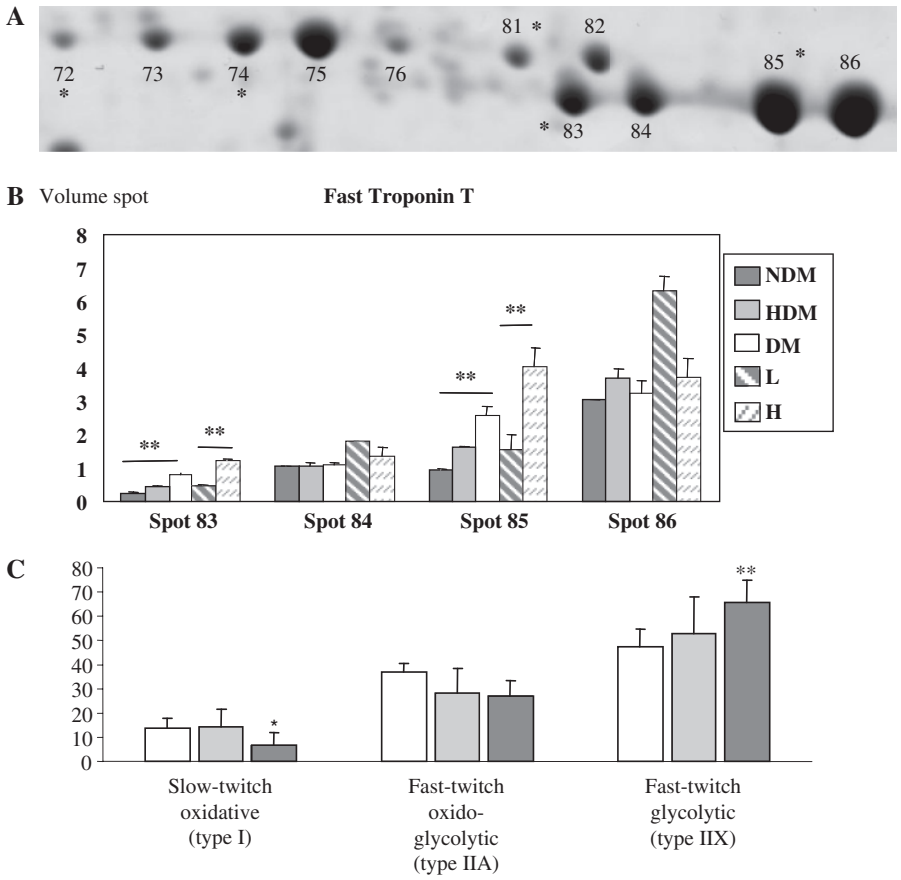

Figure 6: (**A**) Illustration of the isoforms of fast TnT as revealed by Bouley *et al*. [5, 9]. Spots with asterisk originated from exon 16, and the others from exon 17; (**B**) Abundance of different fast TnT isoforms containing either exon 16 (spots 83, 85) or exon 17 (spots 84, 86) in *semitendinosus* muscle from homozygote double-muscled (DM), heterozygote double-muscled (HDM) and non-double-muscled (N) cattle, and in Charolais bulls exhibiting low (L) or high (H) muscle growth rate; (**C**) Percentage of fibres types in homozygote double-muscled (DM, black), heterozygote double-muscled (HDM, grey) and non-double-muscled (N, white) cattle in *semitendinosus* muscle. $^{**}P < 0.01$.

(COX), indicating reduced mitochondrial activity and suggesting lower proportions of oxidative muscle fibres (Figure 6C).

These results show that increased muscle growth potential of monogenic origin (double-muscled) or obtained by divergent selection, was accompanied by changes in abundance of proteins with contractile properties, and to a lesser extent, proteins related to energy metabolism. These changes are consistent with the general observation that increased muscle growth potential of single or multiple gene origin is associated with increased proportions of fast-twitch glycolytic fibres [9, 46–48]. In accordance with this, a study by Hamelin *et al*. [49] dealing with the effects of a quantitative trait locus (QTL) for muscle hypertrophy in four ovine muscles revealed 63 spots differentially expressed between the genotypes and concluded that muscle hypertrophy was associated with higher levels of enzymes involved in glycolytic metabolism.

POST-MORTEM PROTEOME CHANGES

One of the major problems of the meat industry is the variability of technological and sensory meat quality. Meat quality is a complex trait depending on the intrinsic properties of the muscle, such as structural and chemical characteristics of the connective tissue network, intramuscular fat deposition, myofibre size and fibre type composition [50]. It depends further on biochemical processes during *post-mortem* (*p.m.*) transformation of muscle to meat. After bleeding, muscle energy metabolism is modified: nutrients and oxygen are no longer supplied to the muscle, calcium ions may move from the

sarcoplasmic reticulum into the cytoplasm of the muscle cell and activate many pathways including ATPases, and metabolites accumulate. The regeneration of ATP depends first on the degradation of phosphocreatine, which has a half-life of 60–80 min in the *p.m.* muscle [51], the production of ATP from ADP catalyzed by myokinase, and subsequently on anaerobic glycogenolysis. All these processes lead to the accumulation of protons and lactate, leading to acidification of the muscle [52]. In addition to energy metabolism, proteolysis, lipolysis and oxidation also play a major role in the determinism of meat quality; they underlie the *p.m.* 'ageing' of the meat, a process considered to start after death and last for several days or weeks depending on the species. The ageing process allows the tenderisation of the meat and the enhancement of taste [50, 53–56]. Proteins are central in these processes. They are, on the one hand, targets, on the other, mediators of the biochemical reactions. *Post-mortem* proteomic analyses provide important molecular information on related metabolic pathways and help to identify mechanisms underlying muscle transformation to meat and meat quality development.

Several studies explored *p.m.* changes in proteome in pork, bovine and fish (for review, refer [10]) demonstrating that some spots decrease in intensity, while others increase or even appear *de novo* during storage [57]. Part of these changes involves fragmentations of proteins. For example, in bovine *longissimus* muscle, the abundance of fragments of actin, creatine kinase, HSP27 and α-B-crystallin increased, whereas the amount of intact molecules decreased over 14 days of cold storage [58]. Similarly in pig *longissimus* muscle, fragments of actin, TnT, creatine kinase and α-crystallin accumulated during 72 h of cold storage [59, 60]. The rate of protein fragmentation seems further to increase with increased *p.m.* muscle energy metabolism, as shown by the presence of more protein fragments 20 min *p.m.* in muscles with a faster pH decline [61].

The fragmentation of proteins results to a large extent from the action of proteolytic enzymes. It has been known for many years that proteolytic processes lead to the fragmentation of myofibrils and the degradation of myofibrillar proteins including desmin, titin, nebulin and TnT, considered essential in the process of meat tenderisation (refer [53, 62–64]). Proteomic analyses also gave further insight into the process of proteolysis itself [11]. For example, they demonstrated that many subunits of proteasomes 20S and 26S changed during *p.m.* storage of bovine *semitendinosus* but not *longissimus* muscle [65]. Likewise, in an *in vitro* study, different actin, desmin, troponin and tropomyosin fractions were incubated with μ-calpain, allowing quantification of differences in sensitivity to proteolysis between the different protein fractions [66].

Proteome analyses can also be used to study protein changes in meat after preparation for consumption. Such analyses give insight into processes underlying taste development, and also nutritional value or digestibility of meat proteins. A recent study showed modifications of myofibrillar proteins of raw meat and dry-cured hams after 6, 10 and 14 months of ripening. Actin, tropomyosin and MyLC disappeared during the ripening period and were almost completely hydrolysed after 12 months [67]. The majority of proteomic studies in meat science are based on a combination of 2DE and mass spectrometry. However, during *p.m.* storage and cooking, low molecular weight peptides appear and their presence can be directly studied using mass spectrometry techniques. This technique allowed the identification of seven peptides corresponding to five proteins in cooked bovine *pectoralis profundus* muscle. Three of them were known targets of *p.m.* proteolysis (TnT, Nebulin, Cypher protein), while the other two were the procollagen types I and IV [68].

BIOLOGICAL MARKERS OF MEAT QUALITY

Proteomics may help understand biochemical mechanisms underlying meat quality in order to control or predict them. A useful approach is the comparison of proteomes between animals showing relatively high or low values for a specific trait within a population. In one study, pigs were selected for light or dark colour of the *semimembranosus* muscle [69]. Results showed that 24 out of a total of 290 matched protein spots showed different abundance between colour groups. Muscles giving rise to darker meat had increased abundance of mitochondrial enzymes and other proteins involved in oxidative metabolism, and of chaperones, including HSP27, α crystallin and glucose regulated protein 58 kDa, which play a role in stabilisation and repair of proteins. In contrast, muscles giving rise to lighter meat had increased abundance of cytosolic proteins involved in glycolysis, glutathione S-transferase omega (GSTO) and

DJ-1 protein, which have antioxidant properties. GSTO influences further the opening of calcium channels in the endoplasmic reticulum. Overall, these results suggest that muscles giving rise to lighter meat have more glycolytic enzymes, possibly higher intracellular free calcium levels allowing a faster ATP hydrolysis and early *p.m.* pH decline, less repair capacities [69], leading to enhanced protein denaturation and, consequently, lighter meat colour [70, 71].

Muscle characteristics, and consequently meat quality, are influenced by various factors, including rearing systems, gender and genetic background. A study on pigs assessed the relative impacts of these factors and found that gender influenced 40% of the matched spots, against 10% for rearing systems and 4.5% for sire breed [31]. Results showed that female pigs, compared to castrated males, had increased abundance of many enzymes of the glycolytic pathway and decreased abundance of several mitochondrial proteins, suggesting a greater reliance of muscle energy metabolism on carbohydrates in females, and on fatty acid oxidation in castrated males. Effects of Duroc versus Large White sire breed on muscle proteome profile were limited to small changes in the abundance of various glycolytic enzymes, likely because genetic differences between sires were small [31]. In contrast, another study comparing purebred Large White and Meishan pigs showed that 8.3% of the matched spots were influenced by breed and differences were consistent with previous data implying that Meishan pigs rely more on oxidative metabolism and less on glycolytic metabolism than Large white pigs [72]. Finally, compared to indoor reared pigs, outdoor pigs had decreased abundance of glycolytic enzymes, suggesting increased oxidative metabolism [31]. Similarly, compared to indoor concentrate-fed cattle, outdoor grazing cattle showed increased abundance of myoglobin, necessary for oxygen transport, and of enzymes of the tricarboxylic acid cycle pathway, involved in aerobic metabolism, and decreased abundance of enzymes of the anaerobic metabolism, suggesting increased oxidative metabolism in the grazing group [73]. These results are consistent with the observation that outdoor rearing generally results in the production of redder meat [70, 74–77].

Proteomic analyses have also been used to understand better processes underlying tenderness or to provide protein markers predicting tenderness which is one the most important characteristics for consumer satisfaction [55]. One of these studies compared proteomes of the *semitendinosus* or *longissimus* muscles between groups of young Charolais bulls differing in tenderness [78]. Differences between these groups reflected at least partly differences in muscle fibre type composition. Muscles from cattle of the tender group contained more proteins involved in oxidative metabolism (Succinate dehydrogenase (SDH), Malate dehydrogenase (MDH)) or slow contractile properties (slow TnT isoforms), and less proteins associated with fast glycolytic metabolism (LDH, Enolase, Phosphoglucomutase, Triosephosphate isomerase) or fast contractile properties (fast TnT isoforms) [79]. Another study used the same approach on young Blond d'Aquitaine bulls and found also that increased abundance of SDH was the best predictor of higher initial and overall tenderness explaining 66% and 58% of the variability of these palatability traits, respectively [58]. These results are in agreement with earlier results indicating increased tenderness of muscles containing higher proportions of slow oxidative fibres [80]. However, the relationship between muscle fibre type composition and tenderness is complex as tenderness is also influenced by muscle, species, genetic background, nutritional and environmental factors, slaughter conditions and *p.m.* processing (for review, refer [81]).

Increased tenderness was further correlated with the increased presence of fragments of myofibrillar proteins, such as actin, MyHC and MyLC2 in pigs and cattle [59, 82, 83]. In addition, in young Limousin bulls, parvalbumin, involved in calcium metabolism, showed higher abundance, whereas acyl-CoA-binding protein (ACBP), an intracellular carrier of acyl-CoA esters, showed lower abundance in tender muscles [83]. The expression of DNA binding/transcription factor (TCP1), and inositol 1,4,5-triphosphate receptor 1 (IP3R1), involved in calcium channel activity, were lower in low quality grade, less tender beef [82].

Considerable effort is made to understand the mechanisms underlying the process of tenderisation. It has been known for many years that three main cellular proteolytic systems are involved: Ca^{2+}-dependent proteolysis mediated by calpains, lysosomal proteolysis by cathepsins, and proteasome. The relative contributions of these systems to *p.m.* proteolysis remain largely unknown [54, 63, 64, 84]. The proteolytic process depends not only on the presence of Ca^{2+}, but also on the presence of protease inhibitors, pH and oxidative processes which may modify the

structure or configuration of enzymes or target proteins. The complexity of the process is illustrated by prediction models where six variables were needed to explain 70% of the variability of tenderness among Charolais young bulls [85, 86]. These studies showed that increased levels of protease inhibitors, a faster rate of *p.m.* pH decline and low LDH activity are associated with increased meat toughness. The model illustrates that tenderness depends at least partly on the interactive effects of proteolytic processes and *p.m.* energy metabolism [87].

Results of proteomic analyses are in accordance with existing knowledge. For example, caspase 3 and 8, and μ-calpain showed higher levels, and calpastatin (a μ-calpain inhibitor) showed lower levels in young Charolais bulls showing increased tenderness [83]. The interest of the proteomic approach is that it may give better and more exhaustive insight into mechanisms underlying the tenderisation process and identify new protein markers of tenderness. Several proteins of the HSP family were found to be related with tenderness. For example, Charolais bulls with a lower expression of the DNAJA1 gene, in terms of mRNA as well as protein, produced a meat that had better tenderness. The DNAJA1 gene codes for HSP40, a chaperone involved in protein import into mitochondria and a co-chaperone of HSP70. DNAJA1 mRNA levels can explain up to 65% of tenderness variability [88]. Similarly, lower HSP27 levels were found in beef that had greater tenderness, was lighter, and redder, and contained more intramuscular fat, compared to their counterparts [82]. In another experiment, lower levels of HSP27 and HSP p20/122 were also found in the more tender groups of Charolais and Salers young bulls, respectively [83]. In pigs, a slower early *p.m.* pH decline, generally indicative of tenderer meat, was associated with lower levels of HSP72 [3].

The mechanisms underlying the negative relationships between some HSP levels and tenderness could be related to the anti-apoptotic action of HSP. Calpains, serine and cysteine proteases and the ubiquitin–proteasome pathway are known to play a role in *p.m.* proteolysis and the process of tenderisation, but also in apoptosis [89]. It is also known that various HSP, including HSP27 and HSP70 have an anti-apoptotic function [90]. It was further reported that HSP27 protects desmin from calpain proteolysis [91]. Thus, it has been suggested that the tenderising process relies not only on proteolytic but also apoptotic processes and that increased HSP levels may slow down the *p.m.* tenderisation process by limiting *p.m.* proteolytic and/or apoptotic processes [92]. The mitochondria play an important role in initiation of the apoptotic process, as some anti-apoptotic proteins reside in the outer mitochondrial membrane and pro-apoptotic proteins translocate into the mitochondria to form pro-apoptotic complexes [93]. It was recently shown that tenderer meat showed higher abundance of mitochondrial membrane fragments in the early *p.m.* muscle, suggesting an increased degradation of the mitochondrial membrane, indicative of a faster or earlier onset of the *p.m.* apoptotic process [94]. However, opposite results have been reported in young Blond d'Aquitaine bulls where tenderness scores were positively correlated with levels of HSP27 in the early *p.m.* muscle [58]. These contrasting results could be related to variations in extractability of these proteins. A recent study found that small HSP, including HSP27 and αB-Crystallin, disappeared from the soluble muscle extracts and appeared in the insoluble fraction with increasing muscle acidity [95]. These proteins are known to associate with myofibrils during contraction [96]. In accordance with this, Kwasiborski *et al.* [31] and Laville *et al.* [61] observed lower abundance of small HSP in extracts of early *p.m.* muscle characterised by fast pH decline. Similarly, HSP27 and several glycolytic enzymes disappeared from the soluble fraction and appeared in the insoluble fraction between 10 min and 5 days *p.m.* [97]. One mechanism may be protein precipitation, which occurs when the environmental pH approaches the isoelectric point of the protein, around 6 for some HSP27 isoforms [95]. Alternatively, or in addition, due to the higher *p.m.* metabolic activity, HSP may be actively recruited by myofibrillar or denatured proteins or trapped in the acto-myosin complex [31, 61, 95]. In some studies, lower levels of small HSP in muscle extracts may thus reflect reduced solubility or extractability of these proteins, due to faster *p.m.* energy metabolism and pH decline.

Proteomic analyses were also used to understand processes underlying tenderness of pork [97]. Two groups were selected from a larger group on the basis of their tenderness after cooking evaluated by the Warner–Bratzler shear force test (higher values indicate a higher toughness). Proteome analysis of the *longissimus* muscle that obtained 20 min *p.m.* indicated that the tender group was characterised by an increased abundance of adipocyte-specific fatty

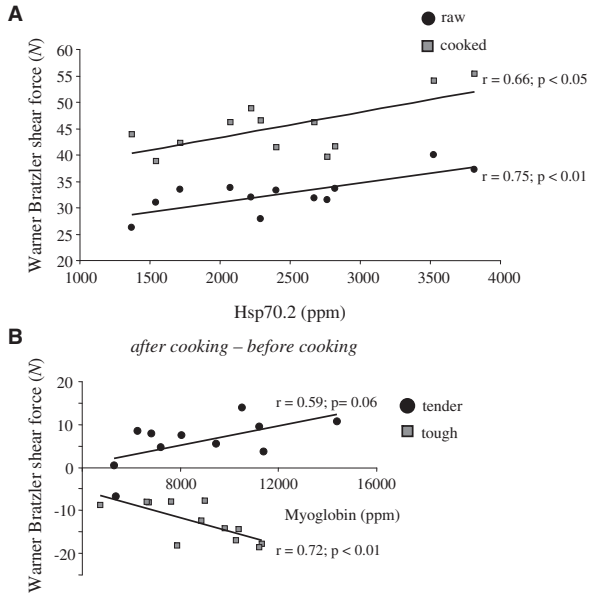

Figure 7: Relationship between intensity of (**A**) a spot identified as HSP70.2 and Warner–Bratzler shear force before and after cooking in a group of pigs selected for low tenderness after cooking and (**B**) a spot identified as myoglobin and the difference between Warner–Bratzler shear forces measured after and before cooking in groups of pigs selected for high or low tenderness after cooking in *longissimus* muscle.

acid-binding proteins suggesting higher levels of adipocytes in meat. Intramuscular fat content and ultimate pH were also higher in the tender compared to the tough group. The tender group showed further increased abundance of proteins involved in protein folding and polymerisation (chaperonin subunit 2, profiling II) suggesting increased protein synthesis. The authors explained the increased tenderness by increased water holding capacity due to higher ultimate pH, and the increased amount of adipocytes which may have contributed to a better solubilisation of collagen during cooking [97]. Further analysis of these data (courtesy of E. Laville, unpublished observations) showed that several proteins could predict tenderness after cooking, but the relationships depended on the treatment group. For example, abundance of HSP70.2 in the early *p.m.* muscle was positively correlated with toughness before and also after cooking for muscles of the tough, but not the tender group (Figure 7A). HSP70.2 has an anti-apoptotic action via the lysosomal pathway [98]. Similarly, the abundance of a spot identified as myoglobin was differently correlated with changes in tenderness following cooking in the two groups: the increase in toughness during cooking was positively related to myoglobin in the tender group, whereas the opposite was found in the tough group (Figure 7B). Myoglobin is an oxygen carrier, indicative of a more oxidative metabolism. Results could suggest that the positive or negative impact of the presence of more oxidative fibres on changes in tenderness after cooking depends on the amount of lipids contained in the muscles. However, further experimentation is needed to fully unravel these relationships, taking also into account other myoglobin spots and proteins indicative of oxidative metabolism. Altogether, these results illustrate the complexity of multifactorial processes involved in the determination of meat tenderness.

Proteomics have also been used to understand better or further characterise various meat quality defects. A well-known defect is a pale, soft and exudative appearance of meat (PSE or PSE-like meat). The phenomenon occurs essentially in pig and poultry species, which have relatively high proportions of fast-twitch glycolytic muscle fibres [turkey: 99–101; chicken: 102, 103; pig: 104, 105]. Pig and poultry PSE meat also show reduced *p.m.* meat ageing [106, 107]. The phenomenon was first described for Pietrain pigs. Later it was found that the phenomenon was related to a single mutation in the ryanodine receptor 1 (RyR1) of the sarcoplasmatic reticulum [108], involved in calcium release. In homozygous carriers of the recessive mutated allele (nn), depending on slaughter conditions, intracellular free calcium levels may be high during and following slaughter, leading to a faster pH decline and consequently denaturation of proteins and reduced water holding capacity. The production of PSE-like meat can also occur in non-carriers, when animals are slaughtered following intense effort or stress [109].

To identify proteins involved in the production of PSE meat, proteomes of pig *semimembranosus* muscle obtained 20 min *p.m.* were compared between homozygous (nn) and heterozygous (Nn) carriers and non-carriers (NN) of the mutation [61]. As expected, nn pigs had a faster early *p.m.* pH decline, but they did not have increased abundance of enzymes of the glycolytic pathway. In accordance with an earlier suggestion [110], it was hypothesised that other glycogenolysis modulating mechanisms are involved in the production of PSE meat. Results showed further that nn muscles contained many more fragments of enzymes and other proteins, suggesting increased

protein degradation during the first 20 min following slaughter. They also showed lower abundance of enzymes involved in aerobic ATP synthesis (pyruvate dehydrogenase, enzymes of the tricarboxylic acid cycle), chaperone-related proteins (HSP27, HSP90, αβ-crystallin) and antioxidants (several proteins of the aldehyde dehydrogenase family). Thus, muscles of the nn genotype appear to be less oxidative and to have less anti-oxidative and repair capacities than NN and Nn genotypes [61]. These results give further insight into the mechanisms underlying the faster *p.m.* metabolism, and increased protein denaturation leading to the production of PSE-like meat. In contrast to PSE pig meat, PSE-like turkey meat showed increased *p.m.* activity of the glycolytic pathway, as indicated by an increased amount of aldolase A and GAPDH compared to normal meat [100].

Early *p.m.* biochemical reactions may have consequences at later stages of the meat production process. For example, raw and cooked ham may present PSE zones appearing the first day *p.m.* [111]. Compared to normal hams, the proteomes of the *semimembranosus* muscle from PSE hams had increased abundance of fragments of creatine kinase. Results showed further a reduced proteolysis of TnT, MyLC1 and α-crystallin in the PSE muscles [111]. The reduced proteolysis of myofibrillar proteins could be the result of precipitation of myofibrillar protein fragments, thus offering protection against proteolysis [111, 112].

EFFECT OF STRESS DURING THE PRE-SLAUGHTER PERIOD

During the pre-slaughter period, animals are subjected to many potentially stressful factors. These stressors may be of physical origin, related to transport or food deprivation, or of emotional origin, due to fear reactions to humans, disturbances in the environment or in social conditions, when animals are separated or mixed with other animals [113].

The effects of stress during the pre-slaughter period are well known. Physical reactions to stress increase metabolic demand and consequently activate muscle metabolism. Stress of emotional origin may stimulate secretion of hormones in the blood, mostly cortisol and catecholamines that exacerbate the effects of muscular activity on muscle metabolism, including muscular glycogen catabolism [114–116]. Stress or exercise immediately before slaughter causes increased muscle metabolic activity which may continue after death, resulting in higher early *p.m.* muscle temperature and lactic acid content and faster pH decline [109, 117, 118]. Exercise and/or stress at any time during the pre-slaughter period, may also cause glycogen depletion in the muscle. Consequently, overall *p.m.* energy metabolism is decreased, resulting in the production of fewer protons and thus, higher ultimate pH of the meat [119–121]. Various sub-chapters above illustrate that the rate and extent of *p.m.* pH decline influence many aspects of meat quality, such as tenderness, colour and water-holding capacity.

The study of the effects of pre-slaughter stress on muscle proteome may help understand the mechanisms underlying the effects of pre-slaughter stress on meat quality. Today, there is still little information. One study on pigs showed that 54% of early *p.m.* temperature variability in the *longissimus* muscle was explained by two proteins: glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an enzyme of the glycolytic pathway, and albumin, involved in the regulation of intracellular calcium levels. Possibly, levels of GAPDH were higher in pigs with warmer muscles because they were physically more active *ante-mortem*. Alternatively, increased GAPDH levels may be indicative of higher proportions of fast-twitch glycolytic muscle fibres, which have a higher capacity to accelerate metabolism than slow-twitch oxidative fibres. The negative correlation between albumin and temperature may be explained by a reduction in *p.m.* metabolic rate due to reduced free intracellular calcium levels [122].

Current data show further that the study of protein mechanisms underlying meat quality, including the effects of stress, needs to take into account animal characteristics. Thus, in castrated males, ultimate pH was best explained by glycerol-3-phosphate dehydrogenase, interpreted as an indicator of total *p.m.* energy metabolism. In females, ultimate pH was best explained by both muscular creatine kinase and dimeric dihydrodiol dehydrogenase, indicators of early and total *p.m.* energy metabolism, respectively [122]. Similarly, slaughter conditions influenced abundance of 19% of the matched protein spots in Duroc sired pigs, and only 4.5% in Large White sired pigs (Terlouw *et al.*, in preparation).

Further studies are needed to understand protein mechanisms underlying the effects of stress on meat quality that are not directly related to energy metabolism. Examples cited above illustrate the

relationship between HSP and various meat quality aspects, including tenderness. It is well known that stress increases the expression of various HSP. For example, HSP72 levels increased in various tissues, including skeletal muscle, following exercise or other challenges such as glycogen depletion, exposure to heat or infection [115, 123, 124]. Proteomics may help determine if HSP mediates part of the effects of stress on sensory meat quality. The mechanisms may differ across muscles. Following a stress, increases in HSP72 and HSP73 showed different time courses in different muscles [125, 126]. In addition, different HSP act together and have multiple functions, involving protein stabilisation and repair, as well as anti-apoptotic and anti-oxidative actions, and may influence meat quality development through different or interacting routes.

Finally, recent work found that pre-slaughter stress may enhance oxidative reactions during ageing of the meat (Gobert *et al.*, submitted for publication). Increased oxidative rate in the meat deteriorates taste, colour and nutritional value as polyunsaturated fatty acid content decreases and reactive oxygen species increase [127, 128]. Today, the underlying mechanisms are unknown, and the interest of utilising proteomic analyses in this context is obvious.

CONCLUSION

These studies show that proteomics may help to understand mechanisms underlying myogenesis, muscle growth and meat quality. Results show that in different livestock species partly the same proteins are involved in muscle ontogeny. Similarly, at least in cattle, proteome profiles of muscles characterised by increased growth potential of different genetic origins show many similarities. Technological and sensory meat quality traits depend on intrinsic characteristics and complex interdependent *p.m.* modifications of the muscle. Various groups of proteins appear to play a role in the development of meat quality, such as enzymes involved in energy metabolism and proteolysis, proteins involved in the control of intracellular calcium levels and protease activities, and chaperone proteins, including HSP involved in stabilisation of myofibrillar proteins or in the regulation of apoptosis. In addition, early *p.m.* modifications strongly influence the subsequent ageing process. Proteomic studies provide further potential protein markers potentially useful to improve meat tenderness or other meat quality traits.

Proteomics is a promising technique providing large amounts of new data which has, however, also its limitations. One reason is that skeletal muscle consists of a heterogeneous population of cells and changes in the concentration of some proteins can result from, or be masked by alterations in the extracellular space of muscle cells. In addition, in muscle cells, MyHC, MyLC, actin, titin and nebulin represent a large percentage of the total protein content, whereas other proteins are only present in very limited amounts, resulting in variations ranging in the order of 10^6. High protein abundance may explain why myofibrillar proteins and associated proteins, particularly small HSP, are often found to be influenced by treatment effects. In addition, transcriptomics determine amounts of all mRNA species in a single assay. In contrast, due to the large range of protein abundance and the high number of different proteins, up to 8–10 times more than mRNA species, proteomics studies only subsets of proteins making comparisons between transcriptomics and proteomics sometimes difficult. Finally, as indicated earlier, like other functional genomic techniques, proteomic analyses result in the creation of large data sets. These data sets may gain in interest if they are combined with other functional genomic or phenotypic data on the same samples, thus creating even larger data sets. Like other tools of functional genomics, the proteomic approach is still in its infancy and new analytical, bioinformatic and biostatistical strategies need to be developed to take the full benefit of these exciting new technologies.

Key Points

- Proteomics is a promising technique providing large amounts of new data.
- Proteomics allows studying large numbers of proteins, including their post-translational modifications.
- Comparative biology of muscle proteome allows the identification of similarity and differences in myogenesis between species.
- Proteomics in livestock allows the identifications of markers of meat quality.

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