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Brigitte Picard [1], Arnaud Cougoul [1], Sébastien Couvreur [2], Muriel Bonnet [1]

[1] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

[2] École Supérieure d'Agricultures, ESA, 55 rue Rabelais - BP 30748 - 49007 Angers Cedex 01, FRANCE

Corresponding author

Dr. Muriel Bonnet: muriel.bonnet@inrae.fr; orcid 0000-0001-7193-3543

ABSTRACT

We aimed to evaluate the relationships between meat or carcass properties and the abundance of 29 proteins quantified in two muscles, *Longissimus thoracis* and *Rectus abdominis,* of Rouge des Prés cows. The relative abundance of the proteins was evaluated using a high throughput immunological method: the Reverse Phase Protein array. A combination of univariate and multivariate analyses has shown that small HSPs (CRYAB, HSPB6), fast glycolytic metabolic and structural proteins (MYH1, ENO3, ENO1, TPI1) when assayed both in RA and LT, were related to meat tenderness, marbling, ultimate pH, as well as carcass fat-to-lean ratio or conformation score. In addition to some small HSP, ALDH1A1 and TRIM72 contributed to the molecular signature of muscular and carcass adiposity. MYH1 and HSPA1A were among the top proteins related to carcass traits. We thus shortened the list to 10 putative biomarkers to be considered in future tools to manage both meat and carcass properties.

Key words: proteomics, beef, marbling, tenderness, ultimate pH, color, carcass properties

1. Introduction

Since several years, genomics and especially proteomics has been used internationally to propose biomarkers of different meat quality traits [1-5]. Most of the studies compared extreme groups of bovines divergent for a quality trait (generally low number of samples, between 5 and 20 per extreme groups according to the studies). These studies have contributed to feed the first step of the biomarker discovery pipeline set up for human medicine purposes [6, 7]. Thus putative biomarkers of tenderness [8], marbling [9-13], ultimate pH (pHu) [14] and of color [15] have been proposed. Most of the results have been obtained on *Longissimus thoracis* muscle that is the muscle with the highest economic value for the beef industry, while few data are also available on *semitendinosus, Rectus abdominis, Psoas major, Triceps brachii, Semimembranosus or Masseter* muscles [16-22].

In order to identify proteins able to discriminate or to predict not only one but at least two meat or carcass traits, we aimed to analyse the relationships between the abundance of 29 proteins and several meat quality and carcass traits, namely tenderness, marbling, pHu, color parameters (L*, a*, b*), the carcass fat-to-lean ratio, the carcass weight and the carcass conformation. The 29 proteins were chosen among a compendium of proteins proposed as biomarkers of several meat quality traits such as tenderness [8, 14], marbling [13], pHu and color parameters (L*, a*, b*) [14, 23] or carcass traits [13], thanks to a combination of approaches, namely meta-analyses, gel-based or gel-free proteomics and immunological detection and quantification of the proteins. To select a limited number of proteins, we have focused on proteins whose abundance were previously related to at least two of these traits. The abundance of the 29 proteins was assayed by reverse phase protein arrays (RPPA) on two muscles: *Longissimus thoracis* (LT) and *Rectus abdominis* (RA) from 48 cows from the Rouge des Prés breed (Protected Designation Origin Maine Anjou). The immunological method RPPA was chosen to quantify the abundance of the 29 proteins on 48 muscles because this method was shown to reach a sensitivity in the ng/mg to µg/mg range of protein abundance compatible with the expected abundances of the 29 selected candidate proteins [24]. The metabolic and contractile properties of the two muscles as well as the rearing practices of the cows [25] and the relationship between the pHu or color traits and the abundance of 18 proteins assayed by a less automated an high throughput immunological methods [14] were first published. From these previous results we selected 48 cows among a larger experiment in order to cover the biological variability of the meat and carcass traits known in European lean breeds. In the current study, targeted proteomics was thus applied to LT and RA muscles in order to consider the contractile and metabolic diversity of the muscles, and the relationships between the abundance of 29 proteins and both meat and carcass traits were considered. To the best of our knowledge this is the first targeted proteomics report considering both several meat and carcass traits in order to understand the shared molecular basis and indicators of these traits of economic importance. As examples of the economic impacts, meat purchasing decisions are influenced by color and tenderness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which was evaluated to annual revenue losses of $1 billion in US [26]. Inconsistency in tenderness

was repeatedly shown to affect consumers' willingness to pay for beef, with an acceptance to pay an average premium of $ 1.84/lb for a tender relative to a tough steak in US [27].

2. Material and method

2.1. Animals, muscles sampling and carcass traits

This study was conducted on 48 Rouge des Prés cows from the French PDO (Protected Designation Origin) chosen from a larger experiment previously described [25] to cover the biological variability of meat and carcass traits as illustrated by the range of values reported for each trait (Table 1). The subsets of the 48 Rouge des Prés cows [14] and the methods used to assay carcass and meat traits [25] were previously reported, and are briefly described in the current paper.

Surveys made it possible to establish the finishing practices of the cows. They were fattened for an average of 108 days (+/- 31), with a minimum of 60 days required by the PDO Maine-Anjou specifications. The finishing period was carried out indoor for all cows with a basic ration composed of hay, haylage or a mixture of both. Concentrate supplementation was on average 7.6 kg per day (+/- 2).

The cows were slaughtered in the industrial abattoir of ELIVIA, Le Lyon d'Angers (France). They were food deprived from the day before slaughter and had free access to water. All the animals were slaughtered in the same conditions, in compliance with French welfare regulations and respecting EU regulations (Council Regulation (EC) No. 1099/2009). They were stunned using captive-bolt pistol prior to exsanguination and dressed according to standard commercial practices. The carcasses were not electrically stimulated. After slaughter, carcasses were chilled and stored at 4°C until 24 h post-mortem. At 24H post-mortem, the *Longissimus thoracis* from the sixth and seventh ribs and a *Rectus abdominis* were excised from the right-hand side of the carcass of each animal 24 h post-mortem and cut, processed or sampled as explained thereafter and in [28].

After slaughter, the hot carcasses were weighted, and the cold carcass weight was automatically calculated by incorporating a loss of 2% on chilling according to the legislation. They were graded by a trained classifier according to the European beef grading system (CE 1249/2008). The carcass conformation was ranked according to the EUROP classification with three levels per class (+, =, −) and was converted into a score value according to a conversion table (Fig. 1). The thickness of the back fat was ranked according to the European classification grid: scores from 1 to 5 with three levels per class (+, =, −). All carcasses were scored between 3= and 3+. The carcass composition in fat, muscle and bone was calculated from the dissection of the fifth rib of the carcass according to the equations developed for Salers breed [29] and detailed by Couvreur et al. [25].

The ultimate pH (pHu) of the two muscles *Longissimus thoracis* (LT, mixed fast oxido-glycolytic muscle) and the *Rectus abdominis* (RA, slow oxidative) were measured on all cows 24h post-mortem using a pH meter (HI9025, Hanna Instruments Inc., Woonsocket, RI, USA) equipped with a glass electrode suitable for meat penetration. The measure was realized between the sixth and seventh ribs for the LT

muscle and at the center of the dorsal part of the RA muscle. The average value used for analysis was obtained from five measurements for each cow.

Twenty-four hours after slaughter the two muscles were collected from the right-hand side of the carcass of each animal. LT was excised between the sixth and seventh ribs and from the dorsal part. Muscle samples were frozen in liquid nitrogen and stored at –80°C for the quantification of proteins and measure of intramuscular fat content (IMF). Other muscle samples of LT and RA were collected 72h after slaughter, were vacuum-packaged and aged for 14 days at +4 °C. Then they were frozen and stored at −20 °C until analysis. These samples were used for Warner-Bratzler shear force (WBSF) measurement.

2.2. Physico-chemical measurements on muscles

The WBSF was measured using a Warner-Bratzler shear device (Synergie200 texturometer) on muscle cut into steaks (20 mm thick) and placed in sealed plastic bags with a porosity of 90 µm (ROBET Matériel, Champagné, France) under vacuum and kept at 4 °C for aging (14 days) before to be frozen at -20°C. Briefly, after thawing 48h at + 4 ° C, the steaks of RA and LT were placed 4h in a thermostated bath at + 18 ° C. They were then cooked using an Infragrill E (Sofraca, France) set at + 320 ° C until the temperature at the heart of the steak reached 55 ° C. From 3 to 5 test pieces (1 * 1 * 4 cm) were taken from the heart of the steak in the direction of the fibers and 3 to 4 repetitions per test tube were carried out. A 1kN load cell and a 60mm/min crosshead speed were used (universal testing machine, MTS, Synergie 200H). Peak load (N) and energy to rupture (J) of the muscle sample were determined.

Intra-muscular fat (IMF) content was measured using an Accelerated Solvent Extractor 200 (Dionex Corporation, Sunnyvale, CA, USA) on 3 repetitions per muscle that were at 24h post-mortem, cut into pieces (1−2 cm cross-section), vacuum packed, and stored at −20 °C until analysis. Briefly 1 ± 0.001 g of meat powder was weighted and placed in a 22 mL extraction cell previously prepared with a cellulose filter and silicon balls. The IMF was extracted with petroleum ether at a temperature of 125 °C and a pressure of 103 bar. The petroleum ether containing IMF was collected and transferred in an evaporation vial previously weighted (± 0.001 g). After 15 min of evaporation, the vial was placed in a drying oven at 105°C for 17h and then weighted (± 0.001 g) to determine the amount of IMF in the meat sample.

Meat color was measured at nine locations on each muscle using a portable spectrocolorimeter (Minolta 508i, Minolta Konica, Japan) on LT and RA after a 30 min of blooming period (24h post-mortem, the day of cutting) at +4 °C [14]. The spectrocolorimeter was calibrated before measurement using its standard white calibration tile (Y = 93.58, x = 0.3150, y = 0.3217). Color coordinates were calculated in the CIELAB system: L* (lightness), a* (green to red color components) and b* (blue to yellow color components). An average value of meat color was calculated from three consecutive measurements per muscle.

2.3. Proteins extraction and quantification using Reverse Phase Protein Array Assay

All the reagents were purchased from Sigma-Aldrich, Saint Louis, USA (MO), except when specified. Both sarcoplasmic and myofibrillar proteins were extracted from frozen muscle samples by crushing the samples in the “Precellys 24” tissue homogenizer (Bertin technologies, Saint Quentin-en-Yvelines, France) according to a method previously described [24] . Briefly, 80 mg of frozen muscle for each animal sample was mixed in a buffer containing 50 mM Tris (pH 6.8), 2% SDS, 5% glycerol, 2 mM DTT, 2.5 mM EDTA, 2.5 mM EGTA, 1x HALT Phosphatase inhibitor (Perbio 78420, Perbio Science, Villebon-sur-Yvette, France), Protease inhibitor cocktail complete MINI EDTA-free (Roche, Meylan Cedex France, 1 tablet/10 mL), 2 mM Na3VO4 and 10 mM NaF. The extracts were then boiled for 10 min at 100 °C, sonicated to reduce viscosity and centrifuged 10 min at 15,000 rpm. The supernatants were collected and stored at−80 °C until analysis. Protein concentrations were determined with a commercial protein assay (Pierce BCA reducing agent compatible kit, Thermo Scientific, Waltham, Massachusetts, United State, ref 23252) with BSA as standard.

The RPPA technique used was adapted from Akbani et al. [30]. Extracted muscle proteins were immobilized on a solid phase with high protein binding capacity per unit area, and revealed with specific antibodies. The antibodies used are described in Table 2. Their conditions of used and their specificity in cattle muscles have been previously tested by western blot as described in Guillemin et al. [31] and in Gagaoua et al. [14]. Briefly an antibody was considered specific against a targeted protein when only one band at the expected molecular weight was detected by western blot, and when any band were observed when the first antibody was removed. Optimal dilution ratios for the first and second antibodies and according to the protein concentration were searched during routine procedures of validation and are reported in Table 2.

Briefly, the extracted samples were coated onto nitrocellulose covered slides (Pierce BCA reducing agent compatible kit, Thermo Scientific, Waltham, Massachusetts, United State) using a dedicated arrayer (Quanterix Corp., Billerica, Massachusetts, United States). Four serial dilutions, from 2000 to 250 µg/ml, and two technical replicates per dilution, were used for each sample. Arrays were labelled with 29 specific antibodies (Table 2) or without primary antibody (negative control), using a Dako Autostainer Plus (Agilent, Santa Clara, United States). The slides were incubated with avidin, biotin and peroxydase blocking reagents (Agilent, Santa Clara, United States) before saturation with TBS containing 0.1% Tween-20 and 5% BSA (TBST-BSA). Slides were then probed overnight at 4 °C with primary antibodies diluted in TBST-BSA. After washes with TBST, slides were probed with horseradish peroxidase-coupled secondary antibody (Jackson Immuno Research Laboratories, Newmarket, UK) diluted in TBST-BSA for 1 h at room temperature. To amplify the signal, slides were incubated with Bio-Rad Amplification Reagent for 15 min at room temperature. After washing in TBST, the slides were incubated with Alexa 647-Streptavidin (Invitrogen Molecular Probes, Thermo Fisher Scientific, Waltham, Massachusetts, United States) diluted in TBST-BSA for 1 hour and washed again in TBST. For staining of total protein, slides were incubated 15 min in 7% acetic acid and 10% methanol, rinsed twice in water, incubated 10 min in Sypro Ruby (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and rinsed again. The processed slides were dried by centrifugation and scanned using aGenePix 4000B microarray scanner (Molecular Devices, San José, California, United States). Spot intensity was

determined with MicroVigene software (VigeneTech Inc, Carlisle, Massachusetts, Unites States). The relative abundances of proteins were determined using NormaCurve [32], a SuperCurve-based method that simultaneously quantifies and normalizes RPPA data for fluorescent background per spot, a total protein stain and potential spatial bias on the slide. Next, each RPPA slide was median centered and scaled (divided by median absolute deviation). Remaining sample loading effects were corrected individually for each array by correcting the dependency of the data for individual arrays on the median value of each sample over all the arrays using a linear regression. The quantitative values for proteins abundances in the present study ranges from -6.4 to 2 arbitrary units, and the real abundance of some of these proteins was estimated between 0.2 to 2500 ng/mg of proteins thanks to an absolute quantification using parallel reaction monitoring (PRM) proteomics in similar samples [24].

2.4. Statistical analysis

The statistical analyses were conducted using R version 4.0.2 (R Core Team, 2020). The data were examined for entry errors and outliers. Normality of data was verified with Shapiro-Wilk tests and visualized with plots. For each muscle, the abundance of the 29 proteins was described with the mean and standard deviation. Differences in protein abundance between muscles were compared with paired Student's t tests with an alpha risk of 5%.

To assess the potential of each biomarker of meat or carcass traits both correlation and Partial Least Squares regressions were realized either for the LT or the RA muscle. The relationships between the 29 proteins assayed in one muscle (either LT or RA) and the quality traits (WBSF, IMF, pHu, L*, a*, b*, fat-to-lean ratio, carcass weight and carcass conformation score) were measured with Pearson's correlation coefficients. Carcass conformation was converted into a score value according to a conversion table (Fig. 1, part B). Carcass conformation was thus treated as quantitative score but also as qualitative levels to confirm the results: the variability between different levels of carcass conformation explained by proteins were measured by ANOVA tests. The relationships were considered significant for P-value <0.05. Partial Least Squares regressions (PLS-R) were performed by quality trait to generate explanatory models using the R package ropls [33]. The PLS model overcomes multicollinearity problems and can handle a high number of variables. The components are constructed to maximize the covariance between the trait and the proteins. The choice of the number of components was based on the Q^2 criterion and permutation tests to avoid overfitting and assess the statistical significance of the models. The Variable Importance in Projection (VIP) was computed to estimate the variability of the response explained by each protein. The mean of squared VIP values being equal to 1, the proteins with a VIP >1 are usually considered as the most influential variables of the model. In this study, all the proteins for which the VIP scores were above a threshold of 1 were considered and then compared to those selected from correlation analyses.

To illustrate global pattern, a multiple factor analysis (MFA) was performed using the R package FactoMineR [34]. MFA is an extension of PCA for data structured by groups. In the current paper, the five groups of variables analysed were: carcass characteristics, meat quality traits of both muscles, pHu

and color of both muscles, LT proteins and RA proteins. This method gives equal weight to each group of variables in the construction of the components so that all groups are represented effectively in the projection. The most relevant proteins based on correlations and PLS analyses were selected to represent the groups of muscle proteins. The result is a projection of each variable onto the compromise to analyze communalities and discrepancies.

3. Results

3.1. Muscle effect on the abundances of the 29 proteins

A muscle type effect was observed for 19 proteins among the 29 (Table 3). The most significantly differentially abundant protein between the two muscles were: ENO1, ENO3, CRYAB, TNNT1, ACTA1, MYH1, ALDH1A1, TRIM72, TTN, HSPA1A, PGK1, TUBA4A. Glycolytic enzymes α-enolase 1 (ENO1), β-enolase 3 (ENO3) and Phosphoglycerate kinase 1 (PGK1), as well as Tripartite motif protein 72 (TRIM72), Myosin light chain 1/3 (MYL1), Heat Shock Protein A8 (HSPA8), Four and a half LIM domains 1 (FHL1), Superoxide dismutase (SOD1) and Titin (TTN) were the most abundant in LT (a mixed fast oxido-glycolytic muscle), all others were the most abundant in the slow oxidative RA muscle from Rouge des Prés cows.

3.2. Correlations and prediction of the different meat and carcass quality traits with the 29 proteins

The Variable Importance in Projection (VIP) within the PLS are shown in Fig. 2, 3 and 4 for each meat or carcass trait. The coefficient and P-Value of Pearson correlations are reported in Supplementary Tables S1 to S6. The Table 4 summarizes all the results of Pearson correlations and PLS VIPs for the 29 proteins and the different meat quality traits and carcass properties analyzed in the current paper.

3.2.1. Shear Force (WBSF)

Among the proteins quantified in LT, 11 proteins with a VIP value >1 were selected by the PLS analysis for the prediction of WBSF values of LT muscle (Fig.2). Among these 11 proteins, ten proteins, were also the most significantly correlated to WBSF (Supplementary Table S1). The proteins the most associated (both highly correlated and with high VIP in the PLS prediction) with WBSF of LT (Table 4) were proteins involved in the glycolytic metabolism (negatively linked), mainly ENO1, but also ENO3, PYGB and PGK1; proteins related to the fast contractile type (negatively) MYH1 and MYL1 or the slow oxidative type (positively) TNNT1. Small HSPs (CRYAB and HSPB6, positively) and structural proteins such as α-actinin 3 (ACTN3, negatively) were also related to WBSF of LT.

Among the proteins quantified in RA, 8 proteins were involved in the PLS prediction of the shear force (Fig.2) but 7 were not correlated to this traits. Of these, Peroxiredoxin 6 (PRDX6), was the only protein that was also positively correlated with RA WBSF (Table 4, Supplementary Table S2), and had the

highest VIP, followed by glycolytic enzymes, mainly ALDOA, ENO3 and TPI1 that were positively related to WBSF, while PYGB, the proteolytic protein µcalpain (CAPN1); the oxidative enzyme Retinal dehydrogenase 1 (ALDH1A1) and the structural protein Tubulin alpha (TUBA4A) were negatively related to RA WBSF.

3.2.2. Intramuscular fat content (IMF)

Among the proteins quantified in LT, as summarized in Table 4, 8 proteins were correlated to IMF and they also had VIP > 1 (see also Supplementary Table S1, Fig.2). The proteins the most positively related to IMF were the small HSP family (CRYAB, HSPB6, DNAJA1), the oxidative enzyme ALDH1A1 and PRDX6. The proteins that were negatively related to IMF were the lipogenic enzyme malate dehydrogenase (MDH1), the structural protein ACTA1 as well as, the cell signalling Tripartite motif protein 72 (TRIM72). So, the fattest LT muscles have high cellular oxidative metabolism and consequently high abundance of small HSP and antioxidant enzymes.

Among the proteins quantified in RA, 6 proteins had a VIP value > 1 for the prediction of IMF values (Fig.2) and were also correlated with the IMF values (Table 4). The proteins the most negatively related to RA IMF were the glycolytic enzymes (ALDOA, TPI1, ENO3); while CRYAB, the lipogenic MDH1 and the slow skeletal muscle troponin T (TNNT1) were positively related to IMF values (Table 4, Supplementary Table S2). So the fattest RA, have slow oxidative properties with high abundance of small HSPs as observed for LT muscle, but also high abundance of lipogenic enzyme and low abundance of glycolytic enzyme that does not occurred in LT muscle.

3.2.3. Ultimate pH, pHu

Among the proteins quantified in LT, 9 proteins had a VIP >1 (Fig. 3) and were also correlated with the pHu of LT (Table 4, Supplementary Table S3). The proteins of a fast and glycolytic metabolism (MYH1, ENO3, TPI1, PGK1 and ENO3) were negatively, while protein of a slow and oxidative metabolism (TNNT1, CRYAB) were positively, related to pHu with VIP value highest than 1.5. Additionally HSPB1 and CAPN1 were also negatively related to pHu of LT (Fig. 3 and Table 4).

Ultimate pH of RA muscle was significantly correlated with 8 proteins (supplementary Table S4) which also had VIP values higher than 1 (Fig. 3 and Table 4). The proteins with the highest scores were the structural protein TTN (positively), the cell signalling protein PARK7 (negatively) and the small HSPB6 protein (positively). Other related proteins were TUBA4A, PRDX6, CRYAB (positively), ACTN3 and SOD1 (negatively) which reveals a link between oxidative stresses, slow and structural properties of the RA muscle and the pHu. We can note that CRYAB was the unique protein positively related to pHu in the 2 muscles.

3.2.4. Color parameters (L, a*, b*)*

290 The Table 4 shows that few proteins are related to color parameters of LT, since 4 proteins were
291 both correlated (Supplementary Table S3) and with a VIP value higher than 1 (Fig.3, Table 4). HSPB6
292 was the most associated with L* parameter followed by CAPN1, and they were negatively related to the
293 lightness. HSPA8 and TPI1 were positively associated to L* parameter of LT. Only CAPN1 and TTN
294 were significantly correlated (negatively, Supplementary Table S3) and having VIP >1 (Fig.3, Table 4)
295 with a* parameter of LT. For the b* parameter, the most related proteins were HSPA8 and SOD1
296 (positively, Fig.3, Table 4, Supplementary Table S3).

297 Among the proteins quantified in RA, 7 proteins were both correlated (Supplementary Table S4) and
298 with a VIP value higher than 1 (Fig.3, Table 4) when the L* parameter was considered. Glycolytic
299 enzymes such as TPI1, ENO3, PYGB, PGK1 and fast glycolytic MYH1, as well as structural proteins,
300 namely ACTA1 and ACTN2 were positively associated with L* parameter of RA. Parameters a* and b*
301 of RA were globally associated (both correlated and with VIP values higher than 1) with the same
302 proteins: mainly TTN, TUBA4A and PYGB that were positively, while HSPB1, DNAJA1 and MYL1 were
303 negatively, linked with these parameters (Fig.3, Table 4, Supplementary Table S4). In RA muscle,
304 interestingly PYGB was related positively with the 3 color parameters. When the 2 muscles were
305 considered, TPI1 was positively linked to the L* parameters in both the LT and RA, while TTN was the
306 unique protein linked to a *and b* parameters, however in an opposite direction; negatively in RA and
307 positively in LT.

308 *3.2.5. Carcass fat-to-lean ratio*

309 Of the 29 proteins quantified, 13 proteins assayed in the LT or RA were both correlated to the
310 carcass fat-to-muscle and had a VIP value higher than 1 (Fig.4, Table 4, Supplementary Table S5). Of
311 these, 4 proteins quantified in LT and RA were related to the carcass fat-to-muscle: CRYAB and
312 ALDH1A1 were positively, while MYH1 and TRIM72 were negatively linked to this carcass trait. In
313 addition, of the proteins quantified in the LT muscle, HSPA8 and MDH1 were negatively, while HSPB6
314 PRDX6 and ACTN2 were positively, associated with the carcass fat-to-muscle ratio. When assayed in
315 RA muscle, HSPA1A and TNNT1 were positively, while glycolytic enzymes ENO3 and PGK1, were
316 negatively associated with the fat-to-muscle ratio (Fig.4, Table 4, Supplementary Table S6).

317

318 *3.2.6. Carcass weight*

319 HSPA1A was the only protein positively related (both significantly correlated and with a VIP value >1)
320 to the carcass weight when assayed both in LT and RA (Fig.4, Table 4, Supplementary Tables S5 and
321 S6). In addition, when assayed in LT, PARK7 was positively, while CAPN1 was negatively, linked to the
322 carcass weight.

323

324 *3.2.7. Carcass conformation*

MYH1 assayed both in LT and RA was the only protein positively related (both correlated and with a VIP >1) to carcass conformation (Fig.4, Table 4, Supplementary Tables S5 and S6). So, the Rouge des Prés cows with the highest abundance of MYH1 in LT and RA, also have the highest conformation score within this population. Measured in RA muscle, this protein explained alone 24% of the variability of carcass conformation (P-Value = 0.001, Fig. 1). Moreover, when assayed in LT, the structural proteins ACTN2 and HSPB6 were positively and negatively related to the carcass conformation. In RA muscle, the abundance of ACTN3 and ENO3, a glycolytic metabolic and structural protein, were positively linked to carcass conformation of Rouge des Prés cows.

3.3. Integrative views of proteins the most frequently associated with meat or carcass traits

The principal relationships were evaluated by a MFA method (Fig. 5), that was complemented by summing the number of occurrences linking a protein to a trait (Table 4). Cows that are characterized by high abundances of proteins related to fast and glycolytic metabolic and contractile properties, namely ENO1, ENO3, TPI1, PGK1, MYH1 measured in the two muscles also have a high carcass conformation and weight, low WBSF and pHu of LT, and low IMF values in RA and LT. These cows were also characterized by low abundances of ALDH1A1, HSPB6 and CRYAB in the two muscles. So, this indicates that the Rouge des Prés cows with the highest muscular glycolytic metabolism are characterized by the highest carcass conformation scores and weight as well as tenderness (lowest toughness/WBSF) at least in LT, which is particularly interesting for the economical value of the carcasses. They have also the lowest pHu, fat-to-muscle ratio of the carcass, muscular IMF, and the highest L* color values for the two muscles. All these carcass and meat properties are strongly related to a molecular signature composed of 7 proteins with 2 abundances profiles : MYH1, ENO1, ENO3, TIP1 with the highest abundances and ALDH1A1, HSBP6, CRYAB with the lowest abundances when the tenderness of LT and the carcass weight and conformation are high. High values of WBSF and phU of LT are mainly associated with high abundance of CRYAB, HSPB6, ALDH1A1 assayed both in RA and LT muscle. The proteins the most positively related to adiposity of muscles and carcass were CRYAB and HSPB6 of the two muscles. The present results thus illustrated that, both multivariate (MFA, PLS) and univariate (correlation) analyses suggest that HSPB6, CRYAB, ALDH1A1, MYH1, ENO3, ENO1, TPI1 are the most strongly related to both carcass and muscle traits. Moreover, the projection of these proteins on the MFA plot confirms their central role (Fig. 5).

4. Discussion

One of the final objectives of this work was to gain insight the genericity of biomarkers, i.e. proteins able to discriminate, or to predict, several meat and carcass traits, which could be integrated into a phenotyping tool for the beef industry. The current study is one of the first depicting the relationships between the abundance of muscular proteins and traits related both to meat and carcass qualities. As expected and as a certificate of the reliability of protein quantification, the protein abundances reported in the current study signed the metabolic and contractile properties of the two muscles. Indeed, the lower abundance of MYH1 in LT than in RA, is in accordance with the two times less IIX fibres in LT than RA

363 in Rouge des Prés cows [25]. Moreover, oxidative activity such as isocitrate dehydrogenase was
364 previously reported higher in RA comparatively to LT [25], in accordance with the present results
365 showing higher abundance of proteins from slow oxidative type such as TNNT1, CRYAB, ALDH1A1 in
366 RA than in LT. Structural proteins such as TUB4A4, ACTA1, ACTN2, were significantly more abundant
367 in RA than in LT, which is coherent with the higher cross sectional area of this muscle comparatively to
368 LT (3599 µm2 in RA versus 2910 in LT; [25]). Lastly, 7 of the 29 investigated proteins were related to
369 the meat and carcass trait which will be discussed with an emphasis on small HSP (HSP20, CRYAB)
370 and glycolytic proteins (MYH1, ENO3, ENO1, PYGB, TPI1) that are the top proteins as ranked in Table
371 4. Additionnally, ALDH1A1 and TRIM72 were more mainly related to the muscular and carcass
372 adiposities, and HSP1A1 to the carcass traits.

373

374 ### *4.1. Opposite relationships of small HSPs and glycolytic proteins are a molecular*
375 ### *signature of both meat and carcass traits*

376 In the current study, positive relationships between the abundances of HSPB6 or CRYAB
377 assayed in RA or LT, and toughness of LT, IMF or pH of these two muscles or the fat-to-lean ratio of
378 the carcass, were concomitant to negative relationships between these traits and the abundances of
379 proteins related to fast glycolytic fibres and glycolytic metabolisms, namely MYH1, ENO3, ENO1, PYGB
380 and TPI1. The current results are in agreement with several studies related to meat tenderness or
381 marbling, however few results are available regarding the fat-to-lean ratio of the carcass, which
382 illustrates the originality of the present work.

383 The current relationships between HSPB6 or CRYAB and meat qualities are consistent with previous
384 studies on tenderness [8], marbling [13], pHu [23, 35] and color [36]. Indeed, CRYAB and HSP20
385 abundances assayed either by non-targeted and targeted proteomics were already linked to the LT
386 toughness/tenderness, pHu, lightness of muscle [14-16, 23] of several breeds. Small Heat Shock
387 Proteins (sHSP) belonging to a large group of chaperone proteins, are abundant in skeletal muscle, and
388 were proposed as implicated in the apoptotic processes in *post mortem* muscle that consequently affect
389 meat quality [35, 37, 38]. They are synthesised to prevent unnecessary apoptosis, to preserve cellular
390 proteins against proteolytic degradation, and thus attempt to maintain cell homeostasis. Recently,
391 Malheiros et al. [39] showed that CRYAB and HSPB6 oxidative damage assayed as the carbonylation
392 of CRYAB and HSPB6, are increased in tender LT from Angus (while the opposite was observed for the
393 glycolytic proteins ENO3 and TPI1). These authors proposed that a lower oxidative damage of HSPs in
394 tough meat could protect myofibrillar protein against the proteolysis, which contributes negatively to the
395 beef tenderness. All these results thus suggest a functional role of CRYAB and HSPB6 to the process
396 of meat tenderisation.

397 Elsewhere, HSPB6 and CRYAB concentrations were reported to be modified during *post mortem* ageing
398 of LD from bull sampled in a New Zealand commercial abattoir [35] or in Angus cross bulls [37], with
399 either positive and negative relationships observed. However, such relationships were not observed in
400 Charolais [40] or Blonde d'Aquitaine [23] young bulls. HSPB6 and CRYAB, were also among the HSP

that were identified to be negatively associated with lightness (L*) in LT of several breeds as previously reviewed [36], and as in the present study.

In Rouge des Prés cows, higher abundance of CRYAB and HSPB6 were recorded in the highest marbled LT when gel-based and gel-free proteomic analyses were done [13], which is thus confirmed by targeted quantitative proteomics in the current study. We previously proposed [13] that high abundance of CRYAB may sustain the high oxidative metabolism of highly marbled muscles, as it is suggested by higher abundance of CRYAB in oxidative compared to glycolytic muscles [41] and with the role of CRYAB in mitochondrial homeostasis and mitoprotection [42]. Moreover, the current study also confirms that high muscular (both in LT and RA) abundance of CRYAB is found in Rouge des Prés cows with high level of fat in the carcass, as previously shown by semi-quantitative proteomics methods [13]. These results are original in bovine, and are consistent with a positive association reported in human models between CRYAB expression in adipose tissue, body mass index and increased levels during adipogenesis [43]. Conversely, we are the first to report a positive relationship between HSPB6 and the fat proportion of bovine carcass. However, a role of HSPB6 in mediating adipocyte function by linking β-adrenergic signalling to PPARγ activity as a negative regulator of adipocyte function was reported in KO mice [44]. Our present results, together with a differential abundance of small HSP, namely HSPB1 and CRYAB in LT from various Canada Beef Grade categories, suggest an involvement of these proteins within the overall meat qualities [45].

The HSPB6 and CRYAB molecular signature of meat WBSF, IMF and carcass adiposity is complemented by a negative relationships between proteins related to glycolytic metabolism and these traits. The myosin heavy chain isoform MYH1 (MyHC IIX protein) is expressed in fast glycolytic fibres, and its abundance in RA or LT muscle of Rouge des Prés cows, was positively associated with carcass conformation score, negatively with carcass fat-to-lean ratio, negatively with shear force (positively with tenderness) of LT muscle, negatively with pHu of LT, positively with lightness of RA.

MYH1 has been found associated with tenderness in many studies, but with different direction according to the muscle [46], the animal type, the gender and breed [8]. The results of the present study confirm a positive relationship between the abundance MYH1 and tenderness (negative when WBSF is considered) in the LT of Rouge des Prés cows as already showed by Couvreur et al. [25] using electrophoresis, and Gagaoua et al. [14] using dot-blot, for MYH1 quantification. The direction of this relation, is similar to that observed in Angus breed characterized as Rouge des Prés, by muscles with more slow oxidative fibres than other French beef breeds, in which the relation between MYH1 and tenderness was negative [46]. The LT muscle of Rouge des Prés has a specific composition of muscle fibres, as it contains few or no IIX fibres [25]. So, our results indicate that when the LT muscle from a breed with slow oxidative muscles contains high proportion of IIX fibres and high intramuscular fat content as observed in Rouge des Prés cows, its tenderness would be higher as already suggested by Picard et al [46]. The negative relationships of MYH1 with IMF is consistent with the literature, clearly identifying MYH1 as a negative biomarker of IMF and fat carcass in different breeds [4].The relationships with pHu and with color parameters are also consistent with other studies [15, 23, 40]. A relationship between MYH1 proportion and muscle mass has been described in the literature [16, 47] in coherence

with the higher carcass conformation and with the negative relation with fat-to-muscle ratio of the carcass observed in the current study.

Of the proteins related to the glycolytic metabolism, 3 proteins involved in the glycolysis were found to be among the top proteins linked with both meat and carcass traits: ENO3, ENO1 and TPI1. ENO3 and ENO1 are two isoforms of the enolase catalysing the reversible conversion of 2-phosphoglycerate to phosphoenolpyruvate in glycolysis, and their abundances are positively correlated with the abundance of MYH1 [48]. The abundance of ENO3 in RA was positively associated with carcass conformation score, negatively with carcass fat-to-lean ratio, negatively with IMF of RA, and positively with lightness of RA. When assayed in LT, ENO3 was associated negatively with shear force (positively with tenderness) and with pHu of LT. In coherence with our results, this glycolytic enzyme was found associated with meat quality and carcass traits namely, tenderness in 11 studies listed in a meta-analysis [8]; color in 8 [36] to 10 [2] studies reviewed recently, pHu [23], marbling and carcass adiposity [13]. ENO3 was proposed as a robust biomarker of color parameters whatever the muscle, probably due to its role in glucose metabolism and cellular protection under hypoxic conditions [36]. Indeed, ENO3 is mainly expressed in adult skeletal muscle cells and was described to have an important role in glucose metabolism and cell protection under hypoxic conditions [49, 50]. Lastly, in the present study, the abundance of ENO3 but also of TPI1 and PGK1 were related to color parameters of RA and LT muscle, in agreement with the glycolytic pathway proposed to be a top pathways involved in bovine meat color [2]. These data are coherent with a high involvement of ENO3 during post-mortem modification of muscle into meat under hypoxic conditions.

Comparatively to ENO3 and MYH1, ENO1 was weakly related to carcass properties, and could be rather considered as a biomarker of meat qualities: tenderness and pHu in LT muscle and color in RA. ENO1 appeared particularly important for LT quality as the VIP value was the highest in the prediction of LT WBSF. This result is coherent with previous data indicating a high role of ENO1 in LT tenderness [31] and its association with tenderness in a meta-analysis [8]. Thus the relationships of ENO3 and ENO1 with meat quality or carcass traits could be explained by the fact that ENO1 has the particularity to be involved in more than one function depending on its cellular and extracellular localization [51]. In addition to its glycolytic activity, ENO1 displays non-glycolytic functions such as cell surface plasminogen binding, maintenance of the mitochondrial membrane stability, transcriptional repressor activity in the nucleus, as well as chaperon and vacuole fusion activity in the cytoplasm [51]. It was described in the literature to be involved in adaptive response of cells to hypoxia. Consequently, its expression should be highly modified post-mortem during the transformation of muscle into meat under hypoxic conditions. Thus, the current results highlight that glycolytic proteins are positively related to the carcass conformation and thus logically negatively related to the fat-to-lean ratio of the carcass, as well as to the marbling of the oxidative RA, and the WBSF mainly of LT.

4.2. In addition to small HSPs and glycolytic enzymes, ALDH1A1 and TRIM 72 signed both muscular and carcass adiposities

The current results confirm the links that we previously hypothesized between CRYAB or ALDH1A1 assayed in LT, and both muscular and carcass adiposities in groups of bovine extreme for these traits [13]. Indeed, in the current study ALDH1A1 assayed both in RA and LT was positively linked to the fat-to-lean ratio of the carcass as well as to the IMF in LT, mainly as CRYAB. ALDH1A1 is a retinal dehydrogenase that irreversibly oxidizes retinaldehyde to retinoic acid [52]. Retinoic acid stimulates, whereas retinaldehyde inhibits lipid deposition in human adipose-derived stem cells [52], which is consistent with the positive relationship between the abundance of ALDH1A1 and lipid deposition both at the muscular and body level. We may also speculate that an ALDH1A1-mediated increase in retinoic acid content has contributed to the increase in the abundance of CRYAB, which is a retinoic acid-responsive gene [52]. The higher abundance of CRYAB may sustain the higher oxidative metabolism of highly marbled muscle, which agrees with the higher abundance of both ALDH1A1 and CRYAB in oxidative compared to glycolytic muscles [41]. As an original result, we report for the first time a negative link between TRIM 72 assayed both in RA and LT and the fat-to-lean ratio of the carcass as well as to the IMF content of LT. TRIM 72 is a muscle-specific mitsugumin 53 that mediates the ubiquitin-dependent degradation of the insulin receptor and insulin receptor substrate 1, comprising a central mechanism controlling insulin signal strength in skeletal muscle [53]. The scarce results linking TRIM72 and adiposity were obtained in mice models of metabolic syndrome, showing a central role of TRIM72 in alleviating insulin resistance [54, 55]. More specifically, low abundances of TRIM72 were concomitant to high level of the muscular glucose transporters GLUT4, which suggested an increase in glucose utilisation in the skeletal muscle, an improvement in systemic insulin sensitivity and a therefore driving less postprandial glucose into hepatic lipogenesis in mice [54]. Whether low abundance of TRIM72 in bovine muscle contributes to maintain high muscle insulin signaling promoting glucose uptake in muscle rather than adipose tissue warrants further investigation. However such TRIM72-mediated insulin sensitivity may be consistent with the higher use of glucose rather than acetate for triglycerides synthesis when intramuscular and body fats are compared [56, 57]. However, this putative pathway may explain the positive link between fast/glycolytic proteins, mainly MYH1 but also ENO3, ENO1 and TPI and the carcass conformation score that grades the muscle mass, while these proteins have a negative link with the fat proportion in the carcass. All these results provide a new evidence for developmental and functional links between muscle and adipose tissue repeatedly reviewed [58-60] and that highlights the balance in nutrient partitioning and thus a priority in the growth and deposition of these two tissues.

4.3. HSP1A1 is positively related to all the carcass traits

Interestingly, HSP1A1 measured in RA and LT muscles was related positively with carcass weight, but also to the fat-to-lean ratio when assayed in RA. Relationships between HSP1A1 and carcass properties were also reported in Blonde d'Aquitaine, Limousine and Angus bulls [61]. Moreover, within a larger population of Rouge des Prés cows, negative and positive correlations between the abundance of HSP1A1 in LT and the carcass conformation score or the fat carcass weight were reported [28], which points out the relation between HSP1A1 abundance in muscle and the carcass adiposity. In humans, there are at least 13 different genes that encode for distinct Hsp70 proteins, but which share a common

domain structure. Of these are the stress-inducible Hsp70 family members encoded for by the *HSPA1A* and *HSPA1B* genes and whose protein products differ by only two amino acids. Due to inconsistencies in nomenclature, the proteins produced by these genes are referred to several different names as simply HSP70, or more commonly in the muscle literature, as HSP72 [62]. Several evidences in models of muscle damage and regeneration demonstrate that the inducible HSP70 is a critical skeletal muscle protein that positively regulates muscle size [62]. Even in non-stressed mice, the increase in muscle hypertrophy was paralleled by an increase in HSP72 abundance in the muscle [63], which is consistent with the current positive relationship with the carcass weight strongly dependent of the muscle mass. Moreover, HSP70 was also shown to play a role in osteogenesis by upregulating the expression of osteogenic genes [64]. Consequently, HSP70 could be associated with carcass traits through an involvement in muscle and skeletal development.

5. Conclusion

With the final aim of developing a molecular test to phenotype meat and carcass traits of economics importance for the beef industry, two main questions must be answered: the muscle to be sampled and a short list of molecules to assay. The current study provides answers to these questions. Compare to the LT, proteins measured in RA are poorly related to shear force of this muscle suggesting that in this muscle the tenderness is related to other characteristics, while the current assayed proteins are strongly related to color parameters. Consequently, we can exclude the use of RA muscle in the future to predict the whole quality of meat and carcass. However, LT muscle has the highest economic value in the carcass, therefore the sampling of this muscle to assess the overall quality of the carcass would lead to economic loss. Secondly, we have shortened the list from 29 to 10 proteins that remain to be quantified in a large population in order to assess the relationship between their abundance and the meat and carcass traits, in a perspective of prediction model establishment. So, future works should focus on the choice of one muscle which can be sampled at slaughter without too much economic loss to quantify the proteins associated with the qualities of meat and carcass. They will also be focused on a tools that quantify protein in a reliable, cost-effective and non-destructive way, as well as in reducing the hands-on steps. Several quantitative methods based on immunoassays or mass spectrometry have been already implemented for human medicine [7], existing biosensors were recently reviewed [1], and the remaining step is to simplify the muscular protein extraction, probably using devices as those produced for plasma proteins [65].

Author contributions

Brigitte Picard: Conceptualization, Funding acquisition, Project administration, Writing - original draft.

Arnaud Cougoul: Data curation; Formal analysis, Visualization, Writing - review & Editing.

Sébastien Couvreur: Funding acquisition, Project administration, Writing - review & Editing.

Muriel Bonnet: Conceptualization, Investigation, Methodology, Validation, Visualization, Writing - original draft.

Declaration of Competing Interest

Authors declare no conflict of interests.

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Table 1

Means, standard deviation (SD) as well as minimal and maximal values for the carcass and meat traits

Traits	Mean	SD	Minimal value	Maximal value
LT muscle traits				
WBSF, N/cm2	46.93	13.85	24.58	82.51
IMF, g/100g DM	4.73	2.42	1.60	13.82
Ultimate pH	5.60	0.11	5.34	5.89
Lightness, L*	39.85	2.20	34.36	43.99
Redness a*	8.96	1.26	6.64	11.77
Yellowness, b*	6.88	1.39	4.02	10.61
RA muscle traits				
WBSF, N/cm2	53.43	13.65	38.60	125.48
IMF, g/100g DM	5.33	2.67	1.28	12.31
Ultimate pH	5.73	0.14	5.46	6.08
Lightness, L*	39.56	1.93	33.60	43.06
Redness a*	6.07	1.03	3.95	8.95
Yellowness, b*	4.49	0.91	2.45	6.50
Carcass traits				
Fat-to-lean ratio, %	0.31	0.10	0.17	0.55
Carcass weight, kg	434.40	29.54	381	553
Conformation score	4.79	0.77	4	6

LT: *Longissimus thoracis*, **RA**: *Rectus abdominis*, **WBSF**: Warner Bratzler Shear Force that is the toughness of the meat, **IMF**: Intramuscular fat content that is the marbling of the meat, color parameters **a*** (green to red color components) and **b*** (blue to yellow color components). Color was assayed after 30 min of blooming period (24h post-mortem, the day of cutting). Conformation Scores (E.U.R.O.P.) are ordinal data which have been treated as quantitative data, the table of conversion used is indicated in the Fig. 1.

758 **Table 2**

759 List of the antibodies (reference and dilution) used to quantify the 29 protein biomarkers using the
760 Reverse Phase Protein Array (RPPA) method.

Protein biomarkers name (*gene*)	**Uniprot ID**	**Monoclonal (Mo) or Polyclonal (Po) antibodies references**	**Antibody dilutions**
Metabolic enzymes			
Malate dehydrogenase (*MDH1*)	**P40925**	Mo. anti-pig Rockland 100-601-145	1/1000
α-enolase 1 (*ENO1*)	**Q9XSJ4**	Po. anti-humanAcris BP07	1/20 000
β-enolase 3 (*ENO3*)	**P13929**	Mo. anti-human Abnova Eno3 (M01), clone 5D1	1/30 000
Retinal dehydrogenase 1 (*ALDH1A1*)	**P48644**	Po. anti-bovine Abcam ab23375	1/500
Triosephosphate isomerase (*TPI1*)	**Q5E956**	Po. anti-human Novus NBP1-31470	1/50 000
Phosphoglycerate kinase 1 (*PGK1*)	**Q3T0P6**	Po. anti-human Abcam ab90787	1/5000
Fructose-bisphosphate aldolase (*ALDOA*)	**A6QLL8**	Po. anti-human Sigma AV48130	1/4000
Glycogen phosphorylase (*PYGB*)	**Q3B7M9**	Po. anti-human Santa Cruz SC-46347	1/250
Heat shock proteins			
αB-crystallin (*CRYAB*)	**P02511**	Mo. anti-bovine Assay Designs SPA-222	1/1000
Hsp20 (*HSPB6*)	**O14558**	Mo. anti-human Santa Cruz HSP20-11:SC51955	1/500
Hsp27 (*HSPB1*)	**P04792**	Mo. anti-human Santa Cruz HSP27 (F-4):SC13132	1/3000
Hsp40 (*DNAJA1*)	**P31689**	Mo. anti-human Santa Cruz HSP40-4 (SPM251):SC-56400	1/250
Hsp70-1A (*HSPA1A*)	**Q27975**	Mo. anti-human RD Systems MAB1663	1/1000
Hsp70-8 (*HSPA8*)	**P11142**	Mo. anti-bovine Santa Cruz HSC70 (BRM22):SC-59572	1/250
Oxidative proteins			
Peroxiredoxin 6 (*PRDX6*)	**P30041**	Mo. anti-human Abnova PRDX6 (M01), clone 3A10-2A11	1/500
Protein deglycase DJ-1(*PARK7*)	**Q99497**	Po. anti-human Santa Cruz DJ-1 (FL-189):SC-32874	1/4000
Superoxide dismutase [Cu-Zn]*(SOD1)*	**P00441**	Po. anti-rat Acris SOD1 APO3021PU-N	1/1000
Structural proteins			
α-actin (*ACTA1*)	**P68133**	Mo. anti-Rabbit Santa Cruz α-actin (5C5):SC-58670	1/1000
α-actinin 2 (*ACTN2*)	**P35609**	Po. anti-human Sigma SAB2100039	1/10 000

α-actinin 3 (*ACTN3*)	**Q0III9**	Po. anti-human Sigma SAB2100040	1/10 000
Myosin light chain 1/3, MLC-1F *(MYL1)*	P05976	Po. anti-human Abnova MYL1 (A01)	1/1000
Myosin heavy chain-I (*MYH7*)	**P12883**	Mo anti-bovine Biocytex 5B9	1/1000
Myosin heavy chain-IIx (*MYH1*)	**P12882**	Mo anti-bovine Biocytex 8F4	1/500
Troponin T, slow skeletal muscle (*TNNT1*)	**Q8MKH6**	Po. anti-human Sigma SAB2102501	1/4000
Titin (*TTN*)	**Q8WZ42**	Mo. anti-human Novocastra NCL-TITIN	1/100
Tubulin alpha-4A chain (*TUBA4A*)	**P81948**	Mo anti-human Sigma T6074	1/1000
Cell death, protein binding and proteolysis			
Tripartite motif protein 72 (*TRIM72*)	**E1BE77**	Po. anti-human Sigma SAB2102571	1/2000
Four and a half LIM domains 1 (*FHL1*)	**Q3T173**	Po. anti-human Sigma AV34378	1/5000
μ-calpain (*CAPN1*)	**P07384**	Mo. anti-bovine Alexis μ-calpain 9A4H8D3	1/500

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Table 3

Muscle effect on the abundance of the 29 proteins. Means and standard deviations (SD) were reported. Differences between the two muscles were measured by paired Student t-tests. The intensity of the gray color is decreasing according to the level of significance.

	LT		RA		Difference RA - LT		Student test
protein	mean	sd	mean	sd	estimated	standard error	p-value
TTN	0.00089	0.4	-1.6	0.57	-1.6	0.092	< .001
TNNT1	0.24	0.51	0.87	0.44	0.64	0.093	**< .001**
ENO3	0.11	0.67	-0.4	0.56	-0.52	0.1	**< .001**
ALDH1A1	0.28	0.67	0.77	0.56	0.49	0.1	**< .001**
ACTA1	0.18	0.5	0.58	0.37	0.4	0.084	**< .001**
ENO1	0.022	0.6	-0.36	0.41	-0.38	0.082	**< .001**
TRIM72	-0.25	0.52	-0.63	0.44	-0.37	0.091	**< .001**
MYH1	-0.56	0.72	-0.15	0.48	0.41	0.1	**< .001**
CRYAB	0.44	0.67	0.77	0.69	0.33	0.087	**< .001**
HSPA1A	0.015	1.4	0.29	1.1	0.28	0.094	**.005**
PGK1	-0.24	0.62	-0.53	0.5	-0.29	0.1	**.006**
TUBA4A	-0.26	1.4	0.33	0.41	0.58	0.21	**.008**
HSPB1	0.61	0.4	0.8	0.42	0.19	0.074	**.013**
MYL1	0.029	0.58	-0.26	0.66	-0.29	0.11	**.016**
HSPA8	0.11	0.49	-0.12	0.43	-0.23	0.092	**.016**
FHL1	0.12	0.58	-0.13	0.53	-0.25	0.11	**.023**
SOD1	0.26	0.52	0.045	0.51	-0.22	0.094	**.027**
PRDX6	0.023	0.51	0.25	0.7	0.23	0.1	**.029**
ACTIN2	0.076	0.78	0.46	1	0.38	0.18	**.042**
DNAJA1	-0.0078	0.61	0.18	0.62	0.19	0.1	.072
MYH7	-0.031	0.67	-0.21	0.64	-0.17	0.096	.075
TPI1	-0.087	0.48	-0.21	0.54	-0.13	0.088	.159
PYGB	-0.47	0.58	-0.33	0.61	0.15	0.11	.191
ALDOA	-0.12	0.53	-0.27	0.55	-0.15	0.11	.207
CAPN1	0.095	0.47	0.2	0.45	0.1	0.091	.259
MDH1	-0.24	0.43	-0.15	0.53	0.095	0.092	.311
ACTIN3	0.15	0.61	0.23	0.62	0.08	0.12	.494
HSPB6	0.64	0.7	0.7	0.58	0.059	0.092	.523
PARK7	-0.22	0.57	-0.2	0.57	0.018	0.11	.867

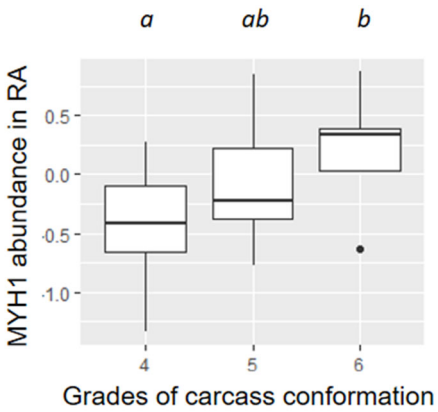
Table 4

Synthesis of the results of Pearson correlation and PLS VIP for the 30 proteins measured in LT and RA, and all the meat quality traits and carcass properties analysed. The positive relationships are in blue, and the negative in orange. The intensity of the color is proportional to the level of the relationship. The stars indicate the level of significance for the correlations: *** <.001, ** <.01, * <.05, . <.1 and level of VIP for the PLS: *** VIP >2, ** VIP >1.6, * VIP >1.2, . VIP >1. #Σp: highlights the sum of correlations, p-values <.1 or VIP values >1 between between a protein and the trait analyzed in order to rank the top proteins.

Protein	WBSF				IMF				pHu				L*				a*				b*				Fat-to-lean ratio				Carcass weight				Conformation score				.#Σp
	LT		RA		LT		RA		LT		RA		LT		RA		LT		RA		LT		RA		LT		RA		LT		RA		LT		RA		
	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	corr	PLS	
MDH1					-*	-**	+**	+***										+*				+*			-***	-**											8
ENO1	-**	-**							-**	-**						+.		+*	-*	-*		+*	-.	-*		-.									+*		13
ENO3	-.	-.		+**			-.	-*	-*	-*					+*	+*		+*		-.							-.	-*							+.	+*	15
ALDH1A1				-*	+**	+**												-.						+.	+**	+*	+*	+**									9
TPI1		-.		+**			-.	-*	-**	-**			+.	+**	+**	+***																-.				+*	12
PGK1	-*	-*						-*	-*	-*					+.	+*											-.	-.									9
ALDOA				+**			-*	-**																								-.					4
PYGB	-.	-*		-.				+.				+.			+.	+.			+*	+*			+*	+**						-*		+*					13
CRYAB	+**	+**			+**	+***	+.	+*	+**	+**	+.	+*		-*											+***	+**	+***	+***									15
HSPB6	+*	+*			+*	+*		+.			+*	+*	-**	-***		-.						-.				+*	+*		+.				+**	-.	-**			17
HSPB1								+.	+*	+*		+.							-.	-.			-*	-*			-.			-*							10
DNAJA1					+**	+**													-.	-.			-*	-*	+.					+*		+*			+.		10
HSPA1A																							-.	-*			+.	+*	+*	+***	+*	+***		+*			9
HSPA8													+.	+**							+.	+**			-.	-.											6
PRDX6			+.	+***	+.	+*		-*			+.	+*							+.			-*			+*	+.				+.							12
PARK7											-*	-**						+*	-.	-.		+*		-.					+*	+**				+*			10
SOD1											-.	-*						+**			+*	+***		-.						+*							7
ACTA1					-.	-.									+**	+***		-*												-.			+*	+***		+*	9
ACTIN2															+*	+*		-.							+*	+.				-.				-*		+.	8
ACTIN3	-*	-*									-.	-*		+*		+.						+*												+**	+.	+*	10
MYL1	-*	-*																	-.	-*			-.	-*													6
MYH7																		+.												-*						-.	3
MYH1	-**	-**							-**	-**					+*	+**								+.	-*	-.	-*	-**				+.	+.	+**	+***	+***	16
TNNT1	+*	+*					+.	+*	+**	+**		+*															+**	+***				+**					10
TTN								+.			+**	+***		-*			-.	-**	+***	+***		-*	+**	+***												+.	12
TUBA4A				-*							+.	+*							+*	+**			+.	+*			+.			-.		+**					10
TRIM72					-*	-*																			-***	-**	-.	-.						+*			7
FHL1																		-*	+*	+**		-*		+.													5
CAPN1				+**					+*	+*			-.	-**		-.	-*	-***				-*							-*	-***							11

Fig. 1. A. MYH1 abundance assayed in the RA muscle of Rouge des Prés cows according to three grades of carcass conformation. **B**. The carcass conformation was ranked according to the EUROP classification with three levels per class (+, =, −) and was converted into a score value according to a conversion table in fifteen grades. A conformation score of 4, 5 and 6 corresponds to a EUROP conformation of O-, O = and O + respectively. **C.** Differences in MYH1 abundance between two grades of carcass conformation.

A



B

1	P-
2	P=
3	P+
4	O-
5	O=
6	O+
7	R
8	R
9	R
10	U
11	U
12	U
13	E
14	E
15	E

C

Conformation grades	MYH1 abundance difference	P- value
5 - 4	0.32	0.056
6 - 4	0.62	0.001
6 - 5	0.30	0.184

Fig. 2. Proteins Variable Importance in Projection (VIP) in PLS regressions of Warner-Bratzler shear force (WBSF) and intramuscular fat (IMF) for the two muscles *Longissimus thoracis* (LT) and *Rectus abdominis* (RA). The protein VIP have been ranked in descending order of VIP. Proteins with a positive effect on the meat traits are shown in blue and those with negative effect in red.

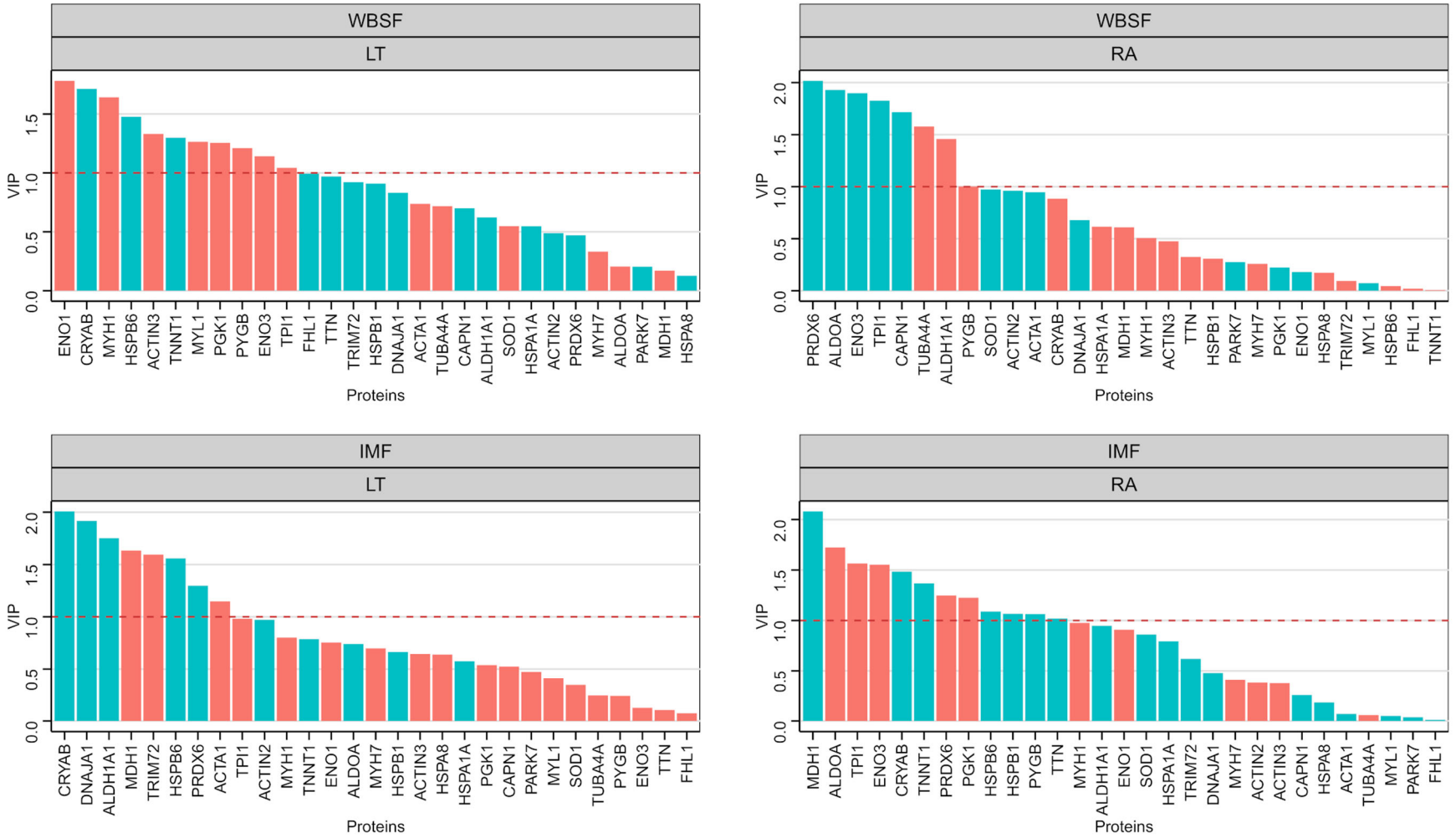


Fig. 3. Proteins Variable Importance in Projection (VIP) in PLS regressions of pHu, L*, a, b color parameters for the two muscles *Longissimus thoracis* (LT) and *Rectus abdominis* (RA). The protein VIP have been ranked in descending order of VIP. Proteins with a positive effect on the meat traits are shown in blue and those with negative effect in red

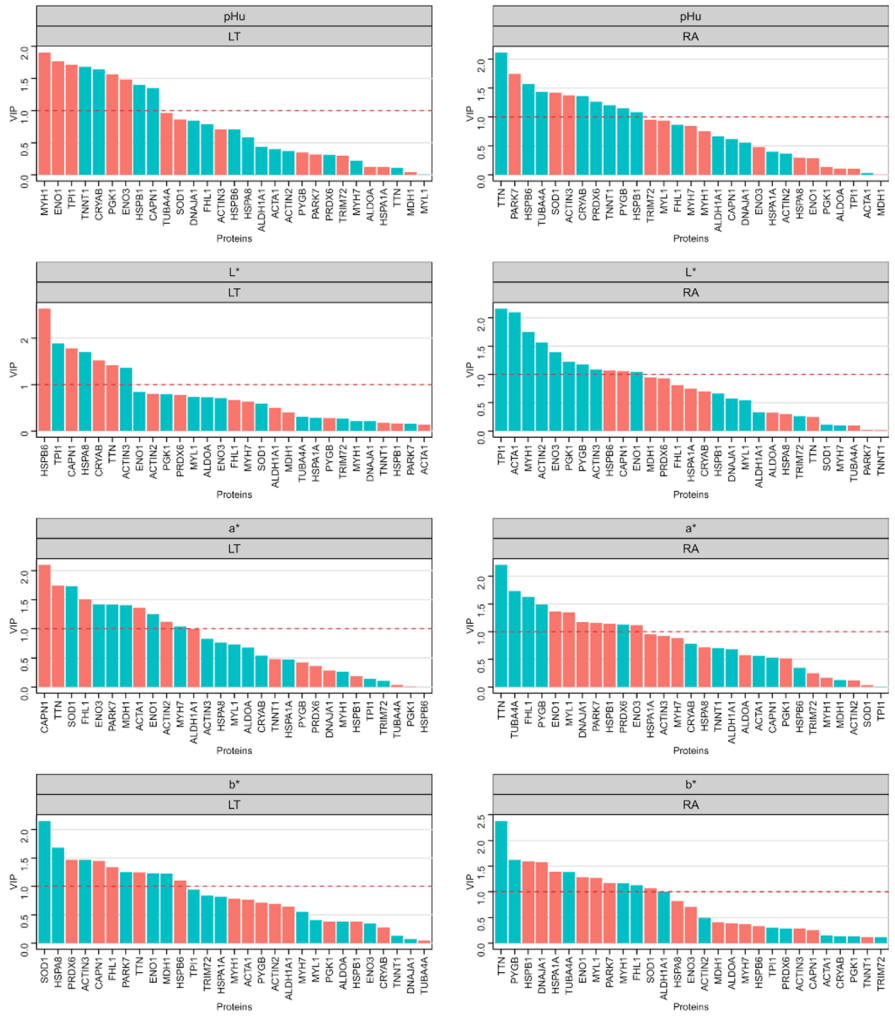


Fig. 4. Proteins Variable Importance in Projection (VIP) in PLS regressions of carcass fat-to-lean ratio, weight and conformation score for the two muscles *Longissimus thoracis* (LT) and *Rectus abdominis* (RA). The protein VIP have been ranked in descending order of VIP. Proteins with a positive effect on the meat traits are shown in blue and those with negative effect in red

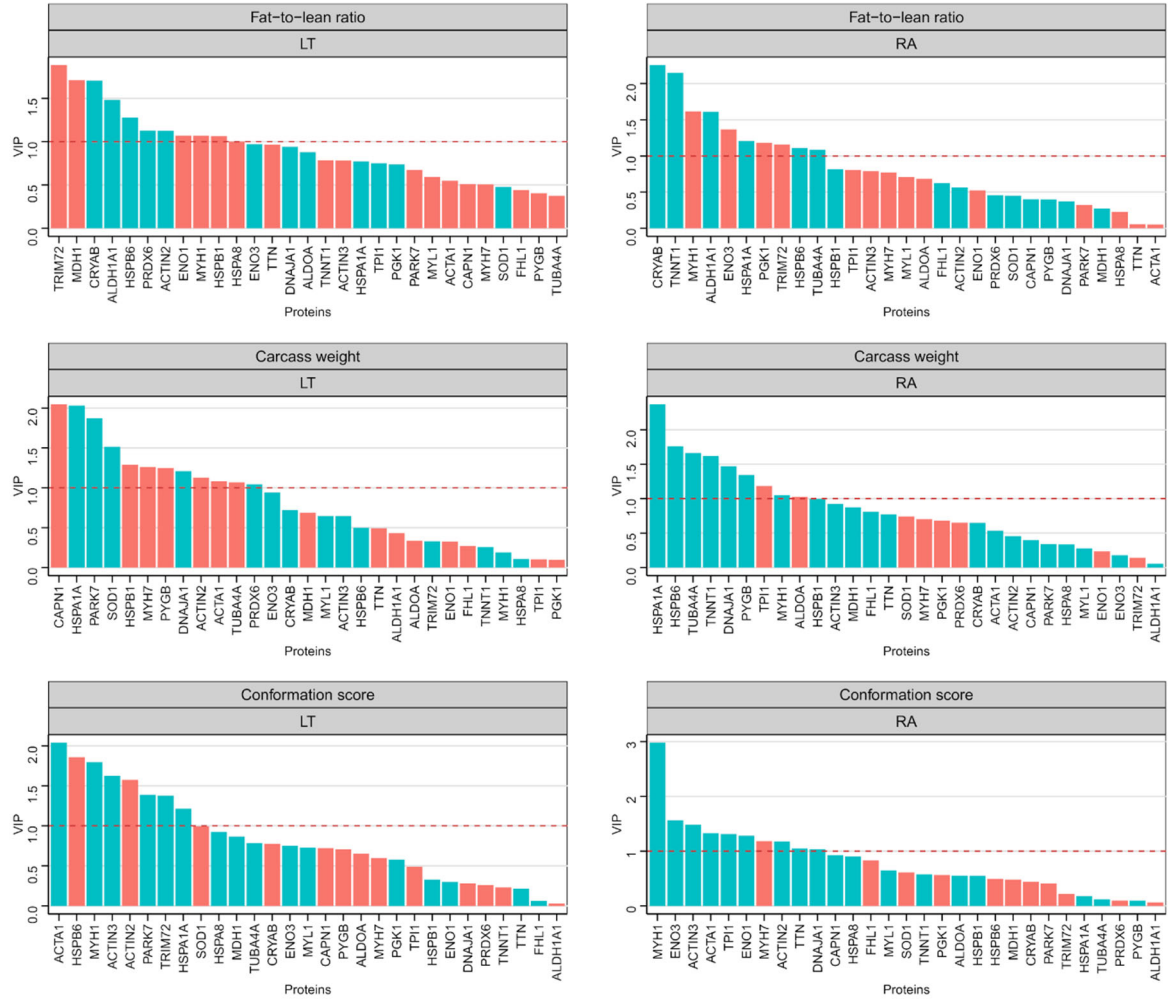


Fig. 5. Multifactorial analysis (MFA) reporting the projection of the proteins the most associated with meat quality and carcass traits when measured in RA or LT muscle, together with the projection of the muscle and carcass traits.