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Relationships between the abundance of 29 proteins and several meat or carcass quality traits in two bovine muscles revealed by a combination of univariate and multivariate analyses

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16 **ABSTRACT**

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

27

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

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34 1. Introduction

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

44 In order to identify proteins able to discriminate or to predict not only one but at least two meat or
45 carcass traits, we aimed to analyse the relationships between the abundance of 29 proteins and
46 several meat quality and carcass traits, namely tenderness, marbling, pHu, color parameters (L^* ,
47 a^* , b^*), the carcass fat-to-lean ratio, the carcass weight and the carcass conformation. The 29
48 proteins were chosen among a compendium of proteins proposed as biomarkers of several meat
49 quality traits such as tenderness [8, 14], marbling [13], pHu and color parameters (L^* , a^* , b^*) [14,
50 23] or carcass traits [13], thanks to a combination of approaches, namely meta-analyses, gel-based
51 or gel-free proteomics and immunological detection and quantification of the proteins. To select a
52 limited number of proteins, we have focused on proteins whose abundance were previously related
53 to at least two of these traits. The abundance of the 29 proteins was assayed by reverse phase
54 protein arrays (RPPA) on two muscles: *Longissimus thoracis* (LT) and *Rectus abdominis* (RA) from
55 48 cows from the Rouge des Prés breed (Protected Designation Origin Maine Anjou). The
56 immunological method RPPA was chosen to quantify the abundance of the 29 proteins on 48
57 muscles because this method was shown to reach a sensitivity in the ng/mg to $\mu\text{g}/\text{mg}$ range of
58 protein abundance compatible with the expected abundances of the 29 selected candidate proteins
59 [24]. The metabolic and contractile properties of the two muscles as well as the rearing practices of
60 the cows [25] and the relationship between the pHu or color traits and the abundance of 18 proteins
61 assayed by a less automated an high throughput immunological methods [14] were first published.
62 From these previous results we selected 48 cows among a larger experiment in order to cover the
63 biological variability of the meat and carcass traits known in European lean breeds. In the current
64 study, targeted proteomics was thus applied to LT and RA muscles in order to consider the
65 contractile and metabolic diversity of the muscles, and the relationships between the abundance of
66 29 proteins and both meat and carcass traits were considered. To the best of our knowledge this is
67 the first targeted proteomics report considering both several meat and carcass traits in order to
68 understand the shared molecular basis and indicators of these traits of economic importance. As
69 examples of the economic impacts, meat purchasing decisions are influenced by color and
70 tenderness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration,
71 which was evaluated to annual revenue losses of \$1 billion in US [26]. Inconsistency in tenderness

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

75 **2. Material and method**

76 *2.1. Animals, muscles sampling and carcass traits*

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

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

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

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

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

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

110 Twenty-four hours after slaughter the two muscles were collected from the right-hand side of the carcass
111 of each animal. LT was excised between the sixth and seventh ribs and from the dorsal part. Muscle
112 samples were frozen in liquid nitrogen and stored at -80°C for the quantification of proteins and measure
113 of intramuscular fat content (IMF). Other muscle samples of LT and RA were collected 72h after
114 slaughter, were vacuum-packaged and aged for 14 days at $+4^{\circ}\text{C}$. Then they were frozen and stored at
115 -20°C until analysis. These samples were used for Warner-Bratzler shear force (WBSF) measurement.

116 *2.2. Physico-chemical measurements on muscles*

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

126 Intra-muscular fat (IMF) content was measured using an Accelerated Solvent Extractor 200 (Dionex
127 Corporation, Sunnyvale, CA, USA) on 3 repetitions per muscle that were at 24h post-mortem, cut into
128 pieces (1–2 cm cross-section), vacuum packed, and stored at -20°C until analysis. Briefly $1 \pm 0.001\ \text{g}$
129 of meat powder was weighted and placed in a 22 mL extraction cell previously prepared with a cellulose
130 filter and silicon balls. The IMF was extracted with petroleum ether at a temperature of 125°C and a
131 pressure of 103 bar. The petroleum ether containing IMF was collected and transferred in an evaporation
132 vial previously weighted ($\pm 0.001\ \text{g}$). After 15 min of evaporation, the vial was placed in a drying oven at
133 105°C for 17h and then weighted ($\pm 0.001\ \text{g}$) to determine the amount of IMF in the meat sample.

134 Meat color was measured at nine locations on each muscle using a portable spectrophotometer (Minolta
135 508i, Minolta Konica, Japan) on LT and RA after a 30 min of blooming period (24h post-mortem, the day
136 of cutting) at $+4^{\circ}\text{C}$ [14]. The spectrophotometer was calibrated before measurement using its standard
137 white calibration tile ($Y = 93.58$, $x = 0.3150$, $y = 0.3217$). Color coordinates were calculated in the
138 CIELAB system: L^* (lightness), a^* (green to red color components) and b^* (blue to yellow color
139 components). An average value of meat color was calculated from three consecutive measurements
140 per muscle.

141

142 *2.3. Proteins extraction and quantification using Reverse Phase Protein Array Assay*

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

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

164 Briefly, the extracted samples were coated onto nitrocellulose covered slides (Pierce BCA reducing
165 agent compatible kit, Thermo Scientific, Waltham, Massachusetts, United State) using a dedicated
166 arrayer (Quanterix Corp., Billerica, Massachusetts, United States). Four serial dilutions, from 2000 to
167 250 µg/ml, and two technical replicates per dilution, were used for each sample. Arrays were labelled
168 with 29 specific antibodies (Table 2) or without primary antibody (negative control), using a Dako
169 Autostainer Plus (Agilent, Santa Clara, United States). The slides were incubated with avidin, biotin and
170 peroxydase blocking reagents (Agilent, Santa Clara, United States) before saturation with TBS
171 containing 0.1% Tween-20 and 5% BSA (TBST-BSA). Slides were then probed overnight at 4 °C with
172 primary antibodies diluted in TBST-BSA. After washes with TBST, slides were probed with horseradish
173 peroxidase-coupled secondary antibody (Jackson Immuno Research Laboratories, Newmarket, UK)
174 diluted in TBST-BSA for 1 h at room temperature. To amplify the signal, slides were incubated with Bio-
175 Rad Amplification Reagent for 15 min at room temperature. After washing in TBST, the slides were
176 incubated with Alexa 647-Streptavidin (Invitrogen Molecular Probes, Thermo Fisher Scientific, Waltham,
177 Massachusetts, United States) diluted in TBST-BSA for 1 hour and washed again in TBST. For staining
178 of total protein, slides were incubated 15 min in 7% acetic acid and 10% methanol, rinsed twice in water,
179 incubated 10 min in Sypro Ruby (Thermo Fisher Scientific, Waltham, Massachusetts, United States)
180 and rinsed again. The processed slides were dried by centrifugation and scanned using aGenePix
181 4000B microarray scanner (Molecular Devices, San José, California, United States). Spot intensity was

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

192

193 *2.4. Statistical analysis*

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

199 To assess the potential of each biomarker of meat or carcass traits both correlation and Partial Least
200 Squares regressions were realized either for the LT or the RA muscle. The relationships between the
201 29 proteins assayed in one muscle (either LT or RA) and the quality traits (WBSF, IMF, pHu, L*, a*, b*,
202 fat-to-lean ratio, carcass weight and carcass conformation score) were measured with Pearson's
203 correlation coefficients. Carcass conformation was converted into a score value according to a
204 conversion table (Fig. 1, part B). Carcass conformation was thus treated as quantitative score but also
205 as qualitative levels to confirm the results: the variability between different levels of carcass conformation
206 explained by proteins were measured by ANOVA tests. The relationships were considered significant
207 for P-value < 0.05. Partial Least Squares regressions (PLS-R) were performed by quality trait to generate
208 explanatory models using the R package ropls [33]. The PLS model overcomes multicollinearity
209 problems and can handle a high number of variables. The components are constructed to maximize the
210 covariance between the trait and the proteins. The choice of the number of components was based on
211 the Q² criterion and permutation tests to avoid overfitting and assess the statistical significance of the
212 models. The Variable Importance in Projection (VIP) was computed to estimate the variability of the
213 response explained by each protein. The mean of squared VIP values being equal to 1, the proteins
214 with a VIP > 1 are usually considered as the most influential variables of the model. In this study, all the
215 proteins for which the VIP scores were above a threshold of 1 were considered and then compared to
216 those selected from correlation analyses.

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

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

225

226 **3. Results**

227

228 *3.1. Muscle effect on the abundances of the 29 proteins*

229 A muscle type effect was observed for 19 proteins among the 29 (Table 3). The most significantly
230 differentially abundant protein between the two muscles were: ENO1, ENO3, CRYAB, TNNT1, ACTA1,
231 MYH1, ALDH1A1, TRIM72, TTN, HSPA1A, PGK1, TUBA4A. Glycolytic enzymes α -enolase 1 (ENO1),
232 β -enolase 3 (ENO3) and Phosphoglycerate kinase 1 (PGK1), as well as Tripartite motif protein 72
233 (TRIM72), Myosin light chain 1/3 (MYL1), Heat Shock Protein A8 (HSPA8), Four and a half LIM domains
234 1 (FHL1), Superoxide dismutase (SOD1) and Titin (TTN) were the most abundant in LT (a mixed fast
235 oxido-glycolytic muscle), all others were the most abundant in the slow oxidative RA muscle from Rouge
236 des Prés cows.

237 *3.2. Correlations and prediction of the different meat and carcass quality traits with the 29* 238 *proteins*

239 The Variable Importance in Projection (VIP) within the PLS are shown in Fig. 2, 3 and 4 for each
240 meat or carcass trait. The coefficient and P-Value of Pearson correlations are reported in Supplementary
241 Tables S1 to S6. The Table 4 summarizes all the results of Pearson correlations and PLS VIPs for the
242 29 proteins and the different meat quality traits and carcass properties analyzed in the current paper.

243 *3.2.1. Shear Force (WBSF)*

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

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

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

259

260 3.2.2. *Intramuscular fat content (IMF)*

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

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

275

276 3.2.3. *Ultimate pH, pHu*

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

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

289 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*

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

333 *3.3. Integrative views of proteins the most frequently associated with meat or carcass traits*

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

354

355 **4. Discussion**

356 One of the final objectives of this work was to gain insight the genericity of biomarkers, i.e. proteins able
357 to discriminate, or to predict, several meat and carcass traits, which could be integrated into a
358 phenotyping tool for the beef industry. The current study is one of the first depicting the relationships
359 between the abundance of muscular proteins and traits related both to meat and carcass qualities. As
360 expected and as a certificate of the reliability of protein quantification, the protein abundances reported
361 in the current study signed the metabolic and contractile properties of the two muscles. Indeed, the lower
362 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 μm^2 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

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

403 In Rouge des Prés cows, higher abundance of CRYAB and HSPB6 were recorded in the highest
404 marbled LT when gel-based and gel-free proteomic analyses were done [13], which is thus confirmed
405 by targeted quantitative proteomics in the current study. We previously proposed [13] that high
406 abundance of CRYAB may sustain the high oxidative metabolism of highly marbled muscles, as it is
407 suggested by higher abundance of CRYAB in oxidative compared to glycolytic muscles [41] and with
408 the role of CRYAB in mitochondrial homeostasis and mitoprotection [42]. Moreover, the current study
409 also confirms that high muscular (both in LT and RA) abundance of CRYAB is found in Rouge des Prés
410 cows with high level of fat in the carcass, as previously shown by semi-quantitative proteomics methods
411 [13]. These results are original in bovine, and are consistent with a positive association reported in
412 human models between CRYAB expression in adipose tissue, body mass index and increased levels
413 during adipogenesis [43]. Conversely, we are the first to report a positive relationship between HSPB6
414 and the fat proportion of bovine carcass. However, a role of HSPB6 in mediating adipocyte function by
415 linking β -adrenergic signalling to PPAR γ activity as a negative regulator of adipocyte function was
416 reported in KO mice [44]. Our present results, together with a differential abundance of small HSP,
417 namely HSPB1 and CRYAB in LT from various Canada Beef Grade categories, suggest an involvement
418 of these proteins within the overall meat qualities [45].

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

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

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

442 Of the proteins related to the glycolytic metabolism, 3 proteins involved in the glycolysis were found to
443 be among the top proteins linked with both meat and carcass traits: ENO3, ENO1 and TPI1. ENO3 and
444 ENO1 are two isoforms of the enolase catalysing the reversible conversion of 2-phosphoglycerate to
445 phosphoenolpyruvate in glycolysis, and their abundances are positively correlated with the abundance
446 of MYH1 [48]. The abundance of ENO3 in RA was positively associated with carcass conformation
447 score, negatively with carcass fat-to-lean ratio, negatively with IMF of RA, and positively with lightness
448 of RA. When assayed in LT, ENO3 was associated negatively with shear force (positively with
449 tenderness) and with pHu of LT. In coherence with our results, this glycolytic enzyme was found
450 associated with meat quality and carcass traits namely, tenderness in 11 studies listed in a meta-
451 analysis [8]; color in 8 [36] to 10 [2] studies reviewed recently, pHu [23], marbling and carcass adiposity
452 [13]. ENO3 was proposed as a robust biomarker of color parameters whatever the muscle, probably
453 due to its role in glucose metabolism and cellular protection under hypoxic conditions [36]. Indeed, ENO3
454 is mainly expressed in adult skeletal muscle cells and was described to have an important role in glucose
455 metabolism and cell protection under hypoxic conditions [49, 50]. Lastly, in the present study, the
456 abundance of ENO3 but also of TPI1 and PGK1 were related to color parameters of RA and LT muscle,
457 in agreement with the glycolytic pathway proposed to be a top pathways involved in bovine meat color
458 [2]. These data are coherent with a high involvement of ENO3 during post-mortem modification of
459 muscle into meat under hypoxic conditions.

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

475

476 *4.2. In addition to small HSPs and glycolytic enzymes, ALDH1A1 and TRIM 72*
477 *signed both muscular and carcass adiposities*

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

508

509 4.3. *HSP1A1 is positively related to all the carcass traits*

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

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

528

529 **5. Conclusion**

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

548 **Author contributions**

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

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

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

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

554

555 **Declaration of Competing Interest**

556 Authors declare no conflict of interests.

557

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565

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749 **Table 1**

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

751

Traits	Mean	SD	Minimal value	Maximal value
LT muscle traits				
WBSF, N/cm ²	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/cm ²	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

752

753 **LT:** *Longissimus thoracis*, **RA:** *Rectus abdominis*, **WBSF:** Warner Bratzler Shear Force that is the toughness of the
754 meat, **IMF:** Intramuscular fat content that is the marbling of the meat, color parameters **a*** (green to red color
755 components) and **b*** (blue to yellow color components). Color was assayed after 30 min of blooming period
756 (24h post-mortem, the day of cutting). Conformation Scores (E.U.R.O.P.) are ordinal data which have been
757 treated as quantitative data, the table of conversion used is indicated in the Fig. 1.

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

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

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767 **Table 3**

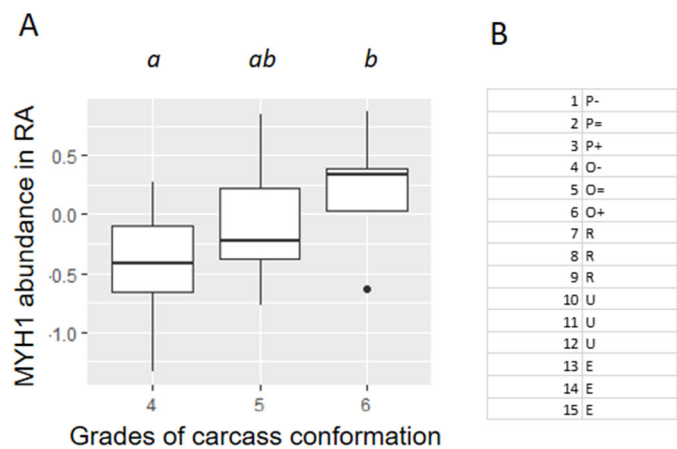
768 Muscle effect on the abundance of the 29 proteins. Means and standard deviations (SD) were
 769 reported. Differences between the two muscles were measured by paired Student t-tests. The
 770 intensity of the gray color is decreasing according to the level of significance.

protein	LT		RA		Difference RA - LT		Student test
	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

780

781 **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
782 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
783 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
784 between two grades of carcass conformation.

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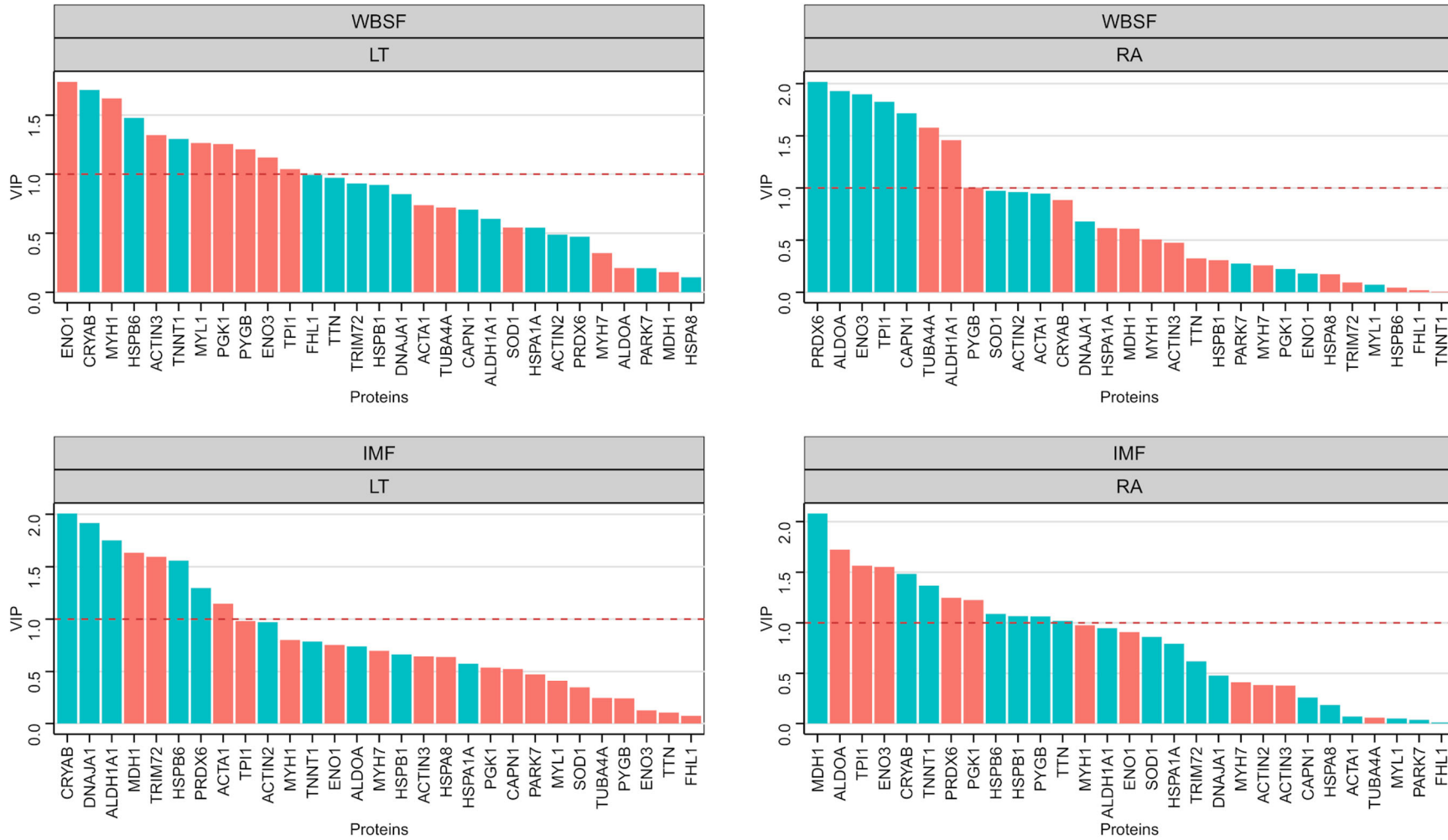
C

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

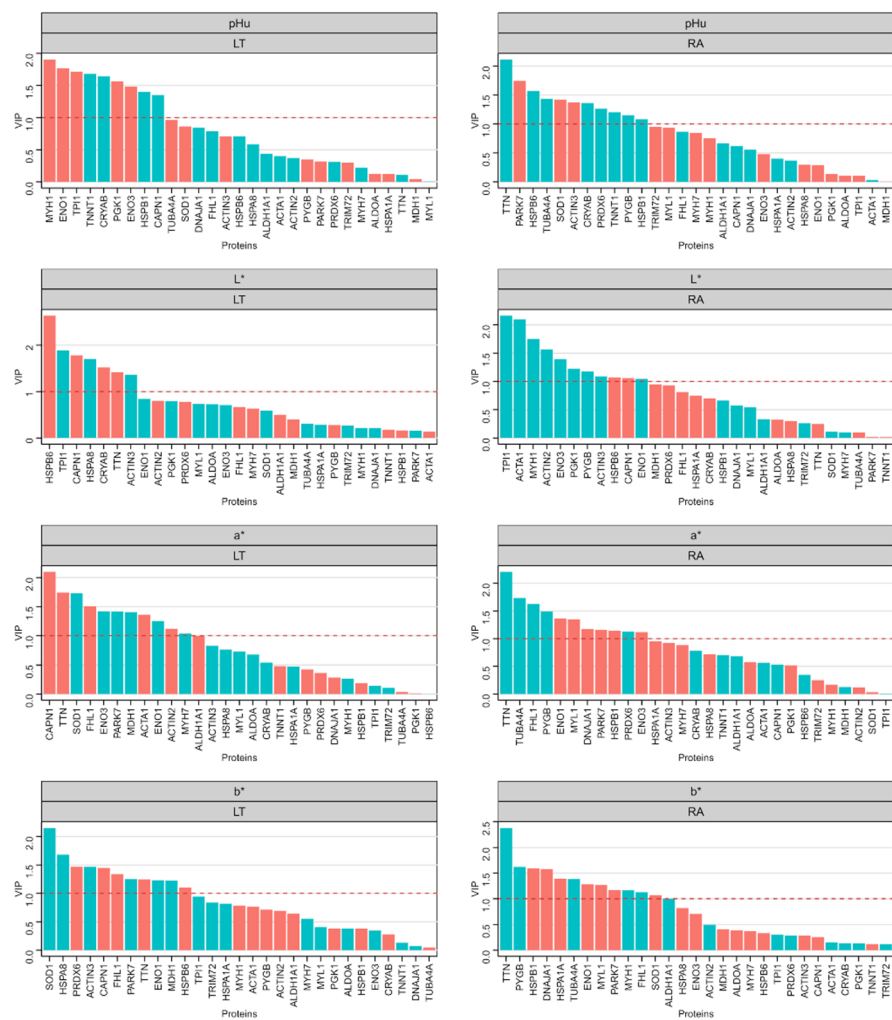
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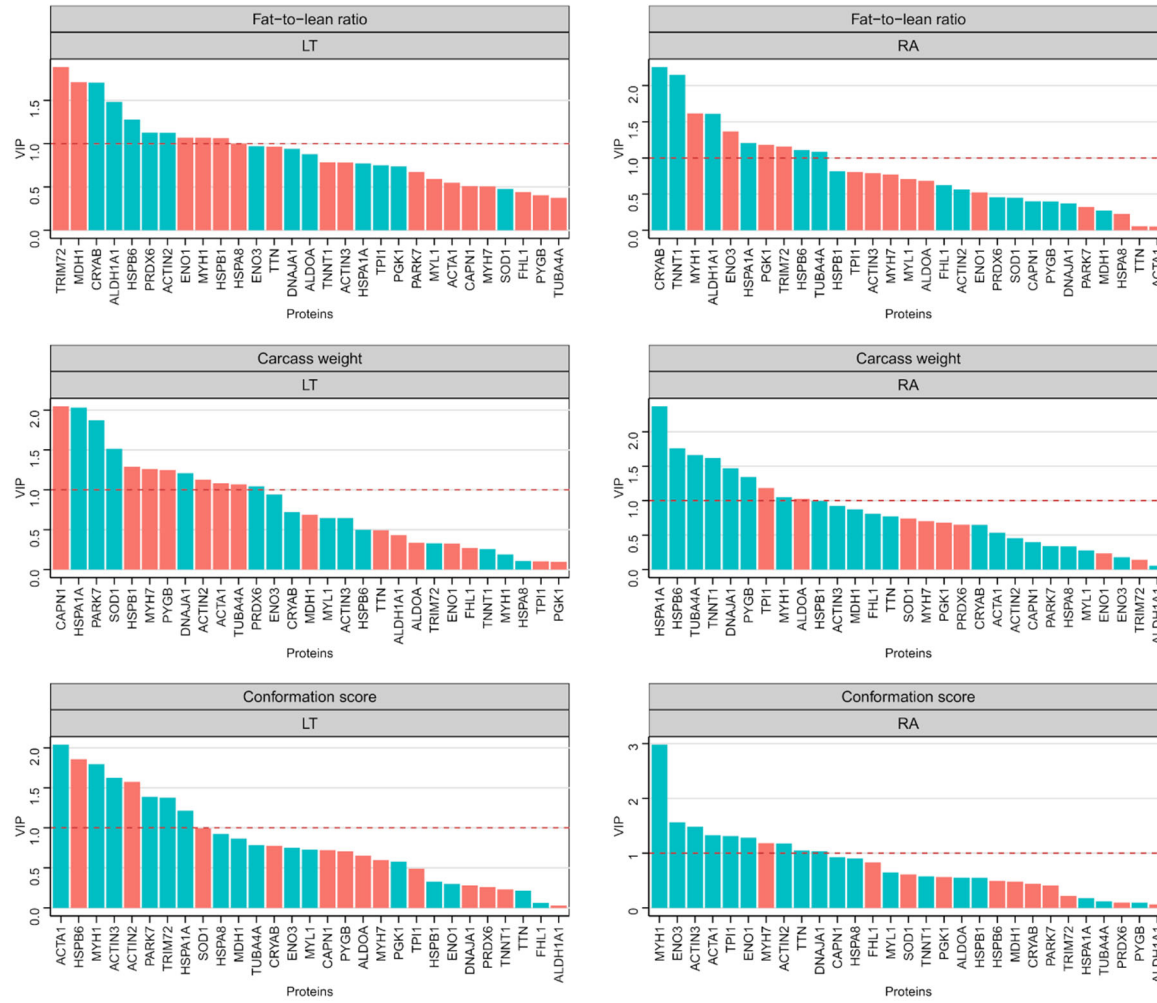
788 **Fig. 2.** Proteins Variable Importance in Projection (VIP) in PLS regressions of Warner-Bratzler shear force (WBSF) and intramuscular fat (IMF) for the two
 789 muscles *Longissimus thoracis* (LT) and *Rectus abdominis* (RA). The protein VIP have been ranked in descending order of VIP. Proteins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



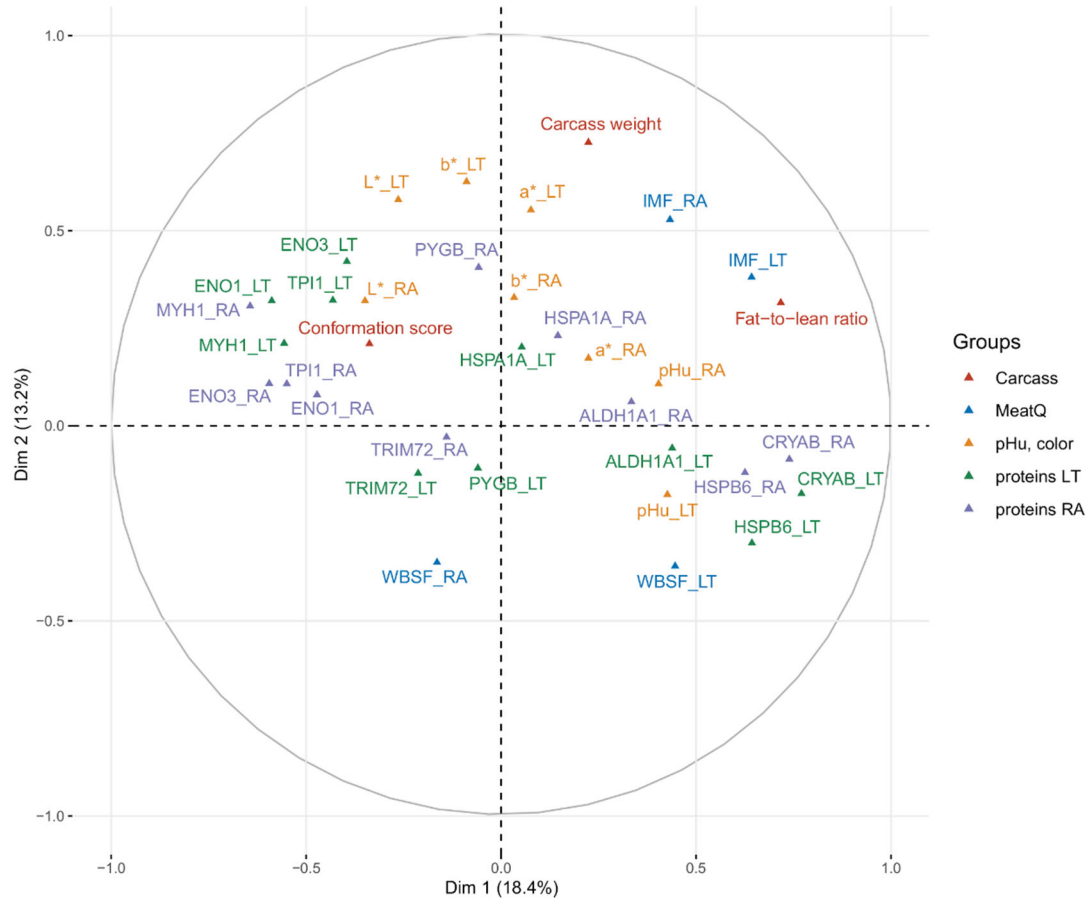
792 **Fig. 3.** Proteins Variable Importance in Projection (VIP) in PLS regressions of pHu, L*, a, b color parameters for the two muscles *Longissimus thoracis* (LT) and
 793 *Rectus abdominis* (RA). The protein VIP have been ranked in descending order of VIP. Proteins with a positive effect on the meat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



796 **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
 797 *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
 798 meat traits are shown in blue and those with negative effect in red



800 **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
 801 LT muscle, together with the projection of the muscle and carcass traits.



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