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1 **Data from the farmgate-to-meat continuum including omics-based biomarkers to better**
2 **understand the variability of beef tenderness: An integromics approach**

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12

13 **Abstract**

14 This study is based on an integromic approach of 71 young bulls' data from farmgate-to-meat
15 continuum including omics-based biomarkers, to understand beef tenderness variability in two
16 muscle cuts that differ by their contractile and metabolic properties. By the means of
17 chemometrics using partial least squares (PLS) and principal component regressions (PCR),
18 important variables from a list of 49 that characterize 4 levels of the continuum (rearing factors
19 – carcass – muscle – meat) were identified to explain tenderness of *Longissimus thoracis* (LT)
20 and *Semitendinosus* (ST) muscles evaluated by sensory panel and instrumental Warner-Bratzler
21 shear force (WBSF). The PLS and PCR analyses validated 16 and 15 variables for LT, and 12
22 and 14 for ST from the whole continuum to explain sensory tenderness and WBSF, respectively.
23 Among the explanatory variables in the 4 models and in line with the role of apoptosis in
24 tenderness determinism, HSP70-1A/B (a heat shock protein) was retained to explain beef
25 tenderness irrespective of muscle and evaluation method. Similarly, dressing percentage from
26 the carcass level was another robust predictor but in a muscle-dependent direction manner.
27 HSP20, ENO3, MyHC-I as three muscle protein biomarkers and dry matter intake (DMI) as a
28 rearing factor, were involved in 3 models to explain beef tenderness. This study highlighted also
29 that several variables were muscle-specific irrespective of the evaluation method of tenderness.
30 For LT muscle, 6 variables including 3 carcass traits (fatness score, fat carcass % and muscle
31 carcass %), 2 muscle biomarkers (HSP70-8 and MyHC-IIx/b) and one meat quality trait (pH_{3h})
32 were found. For ST muscle, 5 variables were validated from which 2 rearing factors (average
33 daily gain and feed efficiency) and 3 structural protein biomarkers (α -actin, MyBP-H and CapZ-
34 β). Finally, for WBSF only, lactate dehydrogenase chain B (LDH-B), was retained positively for
35 LT and negatively for ST muscles. Overall, this trial showed that tenderness of LT and ST
36 muscle-cuts are influenced by variables belonging to the whole continuum with relationships
37 that depend on both the muscle type and the evaluation method. It further highlighted the
38 potential of integromic/chemometric approaches on the farmgate-to-meat continuum data to
39 better understand the sophisticated biological processes that orchestrate the conversion of muscle
40 into meat and tenderness determinism.

41 **Keywords:** Bulls; Meat tenderness; Biological mechanisms; Biomarkers; Chemometrics;
42 Integromics.

43 INTRODUCTION

44 For many years, tenderness was considered by consumers as the most important beef
45 palatability trait affecting overall satisfaction.¹ However, inconsistency in the texture quality of
46 meat at the consumer level has been identified as one of the major problems facing the beef
47 industry. This was described to be due to several intrinsic and extrinsic factors, which are
48 measurable from the farmgate-to-meat levels²⁻⁴, with many of these factors interacting with
49 others. Beef tenderness is believed to be affected by rearing practices (on-farm production
50 factors), such as animal breed, slaughter age, sex, feeding system⁵ and also by muscle structure
51 and composition.⁶ Thus, one of the objectives of beef producers is to develop management tools
52 that enable them to offer a product that meets the high quality requirement of consumers.

53 In this context, numerous national or international research programs were conducted to
54 identify the most relevant factors affecting tenderness in order to develop accurate methods that
55 would interpret and aggregate measures to produce an overall assessment of beef quality. Most
56 of them were carried out after animal slaughter and/or during the ageing period of the carcasses.
57 For example, earlier studies aimed to determine the most important muscle characteristics related
58 to eating sensory meat qualities.⁷ Therefore, muscle fiber characteristics (cross-sectional area,
59 metabolic enzyme activities, proportions of the different muscle fiber types), muscle glycogen
60 content, collagen content and solubility, and the activities of proteases and of their inhibitors
61 during ageing are shown as the most important physiological parameters that determine meat
62 tenderness.⁸ However, conflicting results were reported in the recent literature probably
63 depending on the datasets that differ by many rearing practices. Indeed, the variability explained
64 by these various muscle characteristics alone did not exceed one quarter to one third.^{7,9} This low
65 level of explanation may be due to some unknowns in the different farmgate-to-meat continuum
66 factors that affect tenderness, including gaps in the knowledge of the major biological
67 mechanisms underlying beef tenderness variability.⁶ To avoid this and to be able to propose
68 powerful prediction models, and enhance our understanding of the biological mechanisms,
69 further strategies were applied. Among the proposed methods, high-throughput sequencing (SNP
70 array, RNAseq, etc.) and omic tools were extensively applied during the twenty past years for
71 simultaneous analysis of hundreds of genes, proteins or metabolites as meat tenderness
72 biomarkers.¹⁰⁻¹²

73 Consequently, we have recently reported the potential of combining data obtained by the
74 different approaches described above at the different levels of the continuum such as rearing
75 practices and carcass characteristics to identify beef tenderness classes of young bulls based on

76 decision trees.¹³ Further investigations by means of unsupervised learning tools allowed us to
77 determine which rearing practices and animal traits, as well as live-animal performances and
78 carcass characteristics, need to be improved to reach the targeted market specifications.⁴ The
79 integration of omics data as additional information to those previously mentioned could allow to
80 improve the explanation level of the already existing models. However, to our knowledge there
81 is scarcity in studies from the large literature that have evaluated simultaneously the effects of
82 the whole continuum data, from farmgate-to-meat, including protein biomarkers, on beef
83 qualities. Accordingly, we hypothesized that considering this continuum by a holistic approach
84 based on integromics would characterize sufficiently the main factors driving the desirable beef
85 tenderness for a better understanding of the unknown gaps. This study deals with these aspects
86 and intends to consider several factors of the continuum data by the means of chemometrics, *i.e.*,
87 partial least squares (PLS) and principal component regressions (PCR), as easy and
88 straightforward multivariate methods to relate the multi-dimensional nature of the factors, to beef
89 tenderness. Thus, this trial provides a proof-of-concept concerning the statistical selection of the
90 most influencing factors at different levels of the continuum for accurate understanding of beef
91 tenderness variability. Furthermore, it allowed us to develop an integrated understanding of
92 different mechanisms involved in the development of beef tenderness with a focus on two muscle
93 cuts that differ by their contractile and metabolic pathways.¹⁴⁻¹⁵

94 MATERIAL AND METHODS

95 The study was part of the European FP6 Integrated Project ProSafeBeef (FOODCT-2006-
96 36241) under the INRA reference AQ284.¹⁵ It was carried out under a research program
97 approved by the Ethical Committee of National Institute for Agricultural Research, INRA
98 Auvergne-Rhone-Alpes (ARA), France. This present work is an integrative study of metadata
99 from farmgate-to-meat, which include four levels of the continuum (rearing factors – carcass –
100 muscle – meat) as described in the following sections after a brief summary of the experimental
101 study.

102 *Experimental design: animals, handling and slaughtering*

103 Seventy-one young bulls of three pure breeds (21 Aberdeen Angus, 25 Limousin, and 25
104 Blonde d'Aquitaine) from a balanced experimental design organized in two replicates (2
105 consecutive years, during the spring/summer seasons) as detailed in Gagaoua *et al.*¹⁵⁻¹⁷, were
106 used. None of the animals was double-muscled and they were growth promotant free. At
107 12 months of age, the animals were subjected to a 105 day fattening period until slaughter. The

108 fattening diet given *ad libitum* was based on straw (25%) and concentrate (75%) as detailed in
109 the ProSafeBeef project.⁷ The animals were housed in groups of 4 animals of the same breed in
110 6 × 6 m pens with straw bedding. Before slaughter, all the young bulls were directly transported
111 in a lorry (3 × 2 m) from the experimental farm to the experimental abattoir situated at 1 km from
112 the rearing building, with two bulls of the same home pen per transport to avoid social isolation
113 stress¹⁸. Before slaughter, all animals were fasted for 24 h and had free access to water. After
114 unloading, the animals were slaughtered within 3 min under the standard conditions in the same
115 experimental slaughterhouse of INRA at the Auvergne-Rhône-Alpes Research Center (INRA-
116 Herbipôle, Theix). The animals were stunned using a penetrative captive bolt, prior to
117 exsanguination. Slaughtering was performed in compliance with French welfare regulations set
118 at the level of European Directive (2001/88/EC). The carcasses were not electrically stimulated
119 and they were stored at a maximum temperature of 4 °C (between 2 and 4 °C) up to 24 h *post-*
120 *mortem* according to standard commercial practices.

121 ***Farmgate level: rearing factors practices characterization (q = 8)***

122 The rearing practices of the 71 young bulls were characterized by 8 rearing factors as
123 previously described by Gagaoua *et al.*⁴. They included slaughter age (months), initial body
124 weight before fattening (kg), final body weight (kg), dry matter intake (DMI, kg DM/day), forage
125 and concentrate in per cent (in the DM diet), energy intake (Mcal/day), average daily gain (ADG)
126 for the fattening period, and feed efficiency (ADG/DMI ratio, kg/kg DM).⁴

127 ***Slaughterhouse level: carcass characteristics and composition (q = 8)***

128 The slaughtered animals and thus carcasses were characterized for 8 variables including the
129 weights in kg of the eviscerated animal (empty body weight), cold carcass and 6th rib. The carcass
130 weight was used to calculate the dressing percentage (dressing% = ratio of cold carcass weight
131 to live weight before slaughter, %) and to estimate percentages of muscle and fat tissue in the
132 carcass.¹⁹ The carcasses were further graded under the EUROP carcass grading scheme for
133 carcass conformation and fatness scores. This was done at the slaughterhouse using experts
134 familiar with the EUROP grid (Commission Regulation (EC) 1249/2008). Five conformation
135 classes are defined, represented by the letters E, U, R, O, and P. The scoring consists of a visual
136 assessment of carcass muscling where carcasses graded as ‘E’ have the most muscularity, and
137 this decreases through to ‘P’ which have the least muscularity. European Union regulations allow
138 for 3 subdivisions of each conformation class, high: “+”, medium: “=” and low: “-”. Hence, an
139 incremental scale ranging from 1 to 15 was used, where 1 corresponds to P- (very low muscle

140 development) and 15 to E+ (very high muscle development)¹⁹. At the same time, the fatness
141 score of the carcasses, which describes the amount of fat on the inside and outside of the carcass,
142 was numerically scored from 1 = leanest to 5 = fattest.

143 ***Muscle level: muscle characteristics and protein biomarkers quantification (q = 27)***

144 Muscle samples of *Longissimus thoracis* (LT, mixed fast oxido-glycolytic) and
145 *Semitendinosus* (ST, mixed fast glycolytic) were excised from the right side of each carcass.
146 Overall, four parts of the two samples were taken at 45 min and 24 h *post-mortem* (p-m). The
147 first part of the samples taken at 45 min p-m was subsequently frozen in liquid nitrogen and kept
148 at -80 °C until analyzed for fiber characterization by myosin heavy chains isoforms (MyHC)
149 and for protein extractions for the quantification of tenderness biomarkers by Dot-Blot. The
150 second part was cut in pieces of 1–2 cm cross-section, vacuum packed and stored at -20°C until
151 analyzed for collagen content. Then, the carcasses stored at ~3°C until 24 h p-m were used to
152 sample the third part for meat color measurement of LT muscle only, and the fourth part for
153 sensory evaluation and shear force measurements of the two muscles. For these last part, the
154 samples were cut into steaks (20 mm thick), placed in sealed plastic bags under vacuum and kept
155 at 4 °C for ageing (14 days). They were then frozen and stored at -20 °C until tenderness
156 assessments. All evaluations of muscle characteristics and meat quality were conducted by the
157 same laboratory (INRA, France).

158 ***a. Contractile properties: myosin heavy chains proportions***

159 The contractile properties of the muscles were characterized by the quantification of the
160 myosin heavy chains (MyHC) isoforms according to the electrophoretic method of Picard *et al.*²⁰
161 The electrophoretic separation revealed the existence of MyHC-IIb isoform in few animals (8
162 animals of 71). Consequently, MyHC-IIb percentages were totaled with those of MyHC-IIx
163 creating a new variable “MyHC-IIx+b” (fast glycolytic fibers) as described in Gagaoua *et al.*¹⁵
164 Thus, the proportions of three MyHC isoforms (I, IIa and IIx+b) were considered.

165 ***b. Connective tissue properties***

166 For the analysis of intramuscular connective tissue properties, frozen muscle was
167 homogenized in a household cutter, freeze-dried for 48 h, pulverized in a horizontal blade mill
168 and stored at +4°C in stopper plastic flasks until analyses. For total collagen, about 250 mg of
169 muscle powder were weighed, acid hydrolysed with 10 mL of 6 N HCl, overnight at 110°C in a
170 screw-capped glass tube. Then, the acid hydrolysate was diluted 5 times in 6 N HCl and the
171 subsequent procedure was as previously described and updated by Dubost *et al.*²¹ For insoluble

172 collagen, muscle powder was solubilised according to the method of Hill ²² and hydrolysed
173 according to the same method as for total collagen⁷. Both for total and insoluble collagen each
174 sample was weighed and measured in triplicate and data were expressed in mg of hydroxyproline
175 per g of dry matter (mg OH-pro g⁻¹ DM). Finally, the collagen solubility was determined
176 according to the procedure described by Listrat *et al* ²³.

177 ***c. Protein biomarkers quantification by Dot-Blot***

178 A list of 21 protein biomarkers of meat tenderness chosen according to previous results of our
179 group and data of the literature (see Picard *et al.*¹⁰) were quantified using Dot-Blot technique.¹⁵
180 First, total protein extractions were performed.¹⁵ For that, about 80 mg of frozen muscle at –
181 80°C were homogenized using a Polytron (x22 000) in a denaturation/extraction buffer
182 containing 8.3M urea, 2M thiourea, 1% DTT and 2% CHAPS. After 30 min of centrifugation at
183 10 000g at 8°C, the supernatant was used for protein assay following the method of Bradford²⁴
184 and the relative abundance of 21 protein biomarkers including the entire proteins, their isoforms
185 and protein fragments were quantified.^{14, 25} They belong to 5 biological pathways: 1) heat shock
186 proteins (α B-crystallin, Hsp20, Hsp27, Hsp40, Hsp70-8, Hsp70-1A/B, and Hsp70-Grp75); 2)
187 metabolism (ENO3, LDH-B and MDH1); 3) structure (CapZ- β , α -actin, MyLC-1F, MyBP-H,
188 MYH7, MYH2 and MYH1); 4) oxidative stress (SOD1, Prdx6 and DJ-1) and 5) Proteolysis (μ -
189 calpain). The conditions and suppliers for all primary antibodies dilutions and details of the
190 protocol were exactly according to our previous studies.^{14, 17} The relative protein abundances
191 were based on the normalized volume and expressed in arbitrary units.

192 ***Meat level: sensory and technological meat quality evaluation (q = 8)***

193 ***a. pH values***

194 For muscle pH measurements, biopsies were made from the carcasses (10th rib) at 45 min *p*-
195 *m* (pH_{45min}), whereas pH_{3h} and ultimate pH (pH_u) were measured directly on the right side of the
196 carcasses.¹⁸ Briefly, 2 g of each LT sample excised 45 min after slaughter was immediately
197 homogenized in 18 mL of 5 mM sodium iodoacetate and stored at 4 °C. The pH of the
198 homogenate was measured the following day at 6 °C. For pH_{3h} and pH_u, the pH was recorded
199 directly on the carcass between the 6th and 7th ribs by inserting a glass electrode on five different
200 locations per steak using a Hanna HI 9025 pH/ORP meter suitable for meat penetration (Hanna
201 Instruments Inc., Woonsocket, RI, USA).

202

203 ***b. Color traits***

204 For initial meat color at 24h *p-m* (not color stability), the protocol described by Gagaoua *et*
205 *al.*¹⁹ was applied. A Minolta colorimeter (Minolta CR400, Konica Minolta, Japan) was used to
206 measure the L^* , a^* , and b^* color coordinates of the LT muscle cuts only using illuminant D65
207 (Daylight at noon), 8 mm diameter aperture and a 10° standard observer. The same colorimeter
208 was standardized prior color determination using the ceramic white tile as per the directions of
209 the instrument manual. Six measurements were taken per slice of LT muscle cuts, and the
210 averages were used in the statistical analysis.

211 ***c. Sensory and instrumental tenderness evaluations***

212 For sensory tenderness evaluation, the protocol previously described by our group was used^{16,}
213 ²⁶. Briefly, the meat cuts aged for 14 days were thawed at 5°C for 48 h before cooking at 55°C,
214 the usually used end-point cooking temperature in France^{16, 26}. One hour before sensory
215 assessment, the meat samples were cut into approximately 1.50 cm thick and grilled on a double
216 grooved plate griddle (SOFRACA, Morangis, France) heated to 300°C for 30 min before
217 cooking. The meat cuts were heated for 2 min until the end-points temperature of 55°C in the
218 geometric center of the cut was reached using a temperature probe (Type K, HANNA HI 98704,
219 Newark, USA). After grilling, each grilled sample was cut into 20 mm cubes that were
220 immediately served for sensory assessment to 12 panelists chosen according to the criteria
221 described by Gagaoua *et al.*¹⁶. Thus, sensory panels for each experiment rated the grilled meat
222 samples on a 10 cm unstructured line scale (from 0 to 10) for global tenderness, where 0 refers
223 to extremely tough meat and 10 to extremely tender meat¹⁶. Within each sensory session, scores
224 were averaged across panelists for each sample (animal), and the means were used in the
225 statistical analyses.

226 For objective tenderness, the Warner-Bratzler shear force (WBSF) was measured according
227 to Lepetit and Culioli.²⁷ The WBSF has been widely used in texture evaluation laboratories and
228 still is one of the most commonly used instruments in measuring meat tenderness. Briefly, from
229 the sample cuts cooked as previously described, two to five 1 x 1 x 4 cm cores per steak sample
230 were removed parallel to the longitudinal orientation of the muscle fiber of LT muscle.¹⁴ WBSF
231 was assessed using an Instron 5944 two, three times per core in order to obtain around 10
232 repetitions per sample. Force at rupture during shear compression testing was expressed in
233 N/cm².

234

235 *Statistical analyses*

236 A total of 51 variables at the 4 levels of the continuum from farmgate-to-meat were recorded
237 in this integrative study including 8 rearing factors at the farmgate level; 8 carcass properties at
238 the slaughterhouse level, 27 muscle characteristics at the muscle level and 8 meat quality traits
239 at the meat level (**Tables 1 and 2**). For ST muscle, meat color coordinates (3 variables) were not
240 measured (**Table 3**). In spite of this, these variables were still selected because they are the traits,
241 which have been reported in the large literature during the past three decades to be responsible
242 for animal-to-animal variation in beef tenderness.

243 The data were analyzed using SAS statistical software (SAS 9.3, SAS Institute INC, Cary,
244 NC, USA) and XLSTAT 2018.2 (AddinSoft, Paris, France). Before analysis, raw data means
245 were scrutinized for data entry errors and outliers from the general trends. This was conducted
246 by applying the Smirnov–Grubb's outlier test at 5% levels. After this, a general description
247 analysis was realized by computing means, standard deviations and minimum and maximum
248 ranges of the 51 variables of the whole continuum (**Tables 1, 2 and 3**).

249 For the modeling, a core model was first computed for tenderness measurements (both
250 sensory and instrumental) using multivariate regression analysis under SAS 9.4¹⁹ to consider the
251 fixed effects of breed, replicate and their interaction for each variable in a stepwise manner.
252 Terms in the model and their first order interactions were removed in a stepwise fashion if non-
253 significant ($P > 0.05$). Then, from these data, projections to latent structures by means of partial
254 least squares (PLS) were used to examine how the set of explanatory variables were related to
255 sensory and instrumental beef tenderness in each muscle. The method consists of relating two
256 data matrices X and Y to each other, where in our case the X consists of continuum data except
257 tenderness (X -matrix, 49 variables) and Y is sensory and instrumental tenderness traits (Y -
258 matrix, 2 variables). The filter method with the variable importance in the projection (VIP) was
259 used for variable selection.²⁸ In addition, for variables selection the jack-knife method was
260 included in the PLS regression. Thus, after the first model run including 49 X -variables, those
261 with a $VIP < 1$ were eliminated. A second model was run with the remaining variables and a full
262 cross-validation was performed. The selected variables were those whose regression coefficient
263 was significant ($P < 0.05$) based on principal component regression (PCR). Thus, a PCR was
264 performed on the retained variables for calibration and to identify the direction of the
265 relationships between the X -variables and beef tenderness as recently described by our group.¹⁹
266 PCR and PLS regression are two related families of methods that are often used.

267 **RESULTS**268 *Summary of the PLS and PCR regression models of tenderness traits for LT and ST*
269 *muscles*

270 For sensory tenderness and from the 49 independent variables included in the PLS models,
271 33 (for LT) and 37 (for ST) with a $VIP < 1.0$ were eliminated²⁸. This filter step is important as it
272 improved in the two muscles the explained variation (R^2X : from 0.25 to 0.43 for LT and from
273 0.09 to 0.17 for ST) and the powerful of the link with sensory tenderness (R^2Y : from 0.16 to
274 0.17 for LT and from 0.30 to 0.38 for ST). The final PLS models explained 56% (for LT) and
275 52% (for ST) of the variability of sensory tenderness (**Table 4**). For LT muscle, among the 16
276 variables, 1 was from farmgate level, 4 from slaughterhouse level, 10 were protein biomarkers
277 and 1 variable was a meat trait (**Table 4**). For ST muscle, 12 variables were retained from which
278 3 were from farmgate level, 4 from slaughterhouse level and the remaining 8 variables were
279 protein biomarkers without any variable at meat level (**Table 4**). These retained variables have
280 been validated by PCR regression and gave models with corresponding PCR prediction powers
281 of 68% and 59% of sensory tenderness variability of LT and ST muscles, respectively (**Table**
282 **4**). From these validated predictor variables, 5 of them were common for both muscles to explain
283 sensory tenderness. Among them, the same direction in the two muscles were found, namely for
284 dry matter intake from the farmgate level (positive) and HSP70-1A/B from the muscle level
285 (negative).

286 For WBSF and following the same procedure as for sensory tenderness, 34 (for LT) and 35
287 (for ST) of the 49 independent variables included in the PLS models had a $VIP < 1.0$ and were
288 eliminated. This filter step improved in the two muscles the explained variation (R^2X : from 0.21
289 to 0.31 for LT and from 0.22 to 0.33 for ST) and the powerful of link of the variables with WBSF
290 (R^2Y : from 0.17 to 0.20 for LT and from 0.13 to 0.19 for ST). The final PLS models of WBSF
291 explained similar variations of 57% for LT and 53% for ST compared ($\Delta = +1\%$) to those of
292 sensory tenderness (**Table 5**). For LT muscle and among the 15 variables, 4 were from farmgate
293 level, 4 from slaughterhouse level, 5 were protein biomarkers and 2 were pH traits (**Table 5**).
294 For ST muscle, 14 variables were retained from which 3 were from farmgate level, 1 variable
295 only from slaughterhouse level and the remaining 10 variables were all protein biomarkers
296 without any variable at meat level (**Table 5**). By the same manner as for sensory tenderness, the
297 retained variables have been validated by PCR regression and gave models explaining 66% and
298 63% of WBSF variability of LT and ST muscles, respectively (**Table 5**). From these validated
299 variables, 4 of them were common for both muscles (1 carcass trait and 3 protein biomarkers) to

300 explain WBSF (**Table 5**). Among them, 2 proteins which are HSP70-1B and MyHC-1 (both in
301 arbitrary units) had as expected positive influence on WBSF in the two muscles, explaining the
302 impact on sensory tenderness.

303 ***Robust predictor factors between LT and ST muscles irrespective of tenderness evaluation***
304 ***method (sensory and instrumental)***

305 The PLS models retained for both LT and ST muscles Dressing, % from the slaughterhouse
306 level and HSP70-1A/B from the muscle level, as robust explanatory variables (**Figure 1**) to
307 predict tenderness traits irrespective of the evaluation method (sensory panel or instrumental
308 method. A variable was considered robust if it existed for all the possible situations and included
309 in all the regression explanatory models. In LT muscle, dressing % was negatively related with
310 sensory tenderness and positively with WBSF and the inverse was observed in ST muscle
311 (**Tables 4 and 5**). However, HSP70-1A/B, regardless of the muscle type, was negatively related
312 with sensory tenderness and positively with WBSF in both muscles.

313 ***Common predictor variables by muscle type irrespective of tenderness evaluation method***

314 In LT muscle and from the 16 and 15 variables that were retained in the PLS regression
315 models to explain sensory tenderness and WBSF respectively, 10 variables from the whole
316 continuum were common between the two evaluations of tenderness (**Figure 1**). They were dry
317 matter intake at the farmgate level; 4 carcass characteristics including fatness score, muscle
318 carcass %, fat carcass % and dressing %; 4 protein biomarkers including HSP70-8, HSP70-1A/B,
319 MyHC-I (in AU) and MyHC-IIx+b (%) and finally pH_{3h} from the meat level.

320 In ST muscle and irrespective of the evaluation method of tenderness, 9 variables from the 12
321 and 14 retained variables to explain sensory tenderness and WBSF respectively, were common
322 (**Figure 1**). They were representative of 3 levels only of the continuum including average daily
323 gain and feed efficiency at the farmgate level, dressing % at the slaughterhouse level, and 6
324 biomarkers (CapZ- β , HSP70-1A/B, HSP20, ENO3, α -actin and MyBP-H) at the muscle level.

325 **DISCUSSION**

326 The main objective of the present study was to determine in two muscles of young bulls that
327 differ in their contractile and metabolic properties¹⁴⁻¹⁵ the relationships between beef tenderness
328 traits and a list of factors from the four levels of the continuum from farmgate-to-meat, to allow
329 better understanding of tenderness variability.^{2, 4} Thus, this study aimed to sort through the
330 continuum data, the variables that are the most relevant to explain beef tenderness evaluated by

331 a sensory panel and measured using an instrumental method. The factors identified in this trial
332 to be of paramount importance to control the variation in meat tenderness, should be considered
333 as critical control points for optimal carcass management. Accordingly, the findings of this study
334 identified that different factors and mechanisms underlie beef tenderness, some of which are
335 robust and common between the two muscles whatever the evaluation method of beef tenderness;
336 whereas others are dependent of the type of muscle or of the tenderness evaluation method. The
337 muscle specificities reveal new insights that would help the development of generic prediction
338 tools in the future. In the following sections are discussed the main results of this study by
339 discussing i) the robust predictor variables whatever the muscle and tenderness evaluation, ii)
340 the variables that were retained in three situations, iii) the variables that were specific for each
341 muscle irrespectively of the evaluation method and finally iv) a brief description of those
342 variables that were common for LT and ST muscles regardless of the evaluation method of
343 tenderness.

344 ***Robust predictor variables irrespective of the muscle and tenderness evaluation method***

345 *HSP70-1A/B a robust protein biomarker*

346 In agreement with the role of apoptosis in tenderness⁶, the first interesting variable that was
347 in this trial strongly retained to explain beef tenderness irrespectively of muscle type and
348 evaluation method was HSP70-1A/B (**Figure 1**). HSP70-1A/B is a member of the heat shock
349 proteins (HSPs) that is localized in the cytoplasm and nucleus of muscle fibers.²⁹⁻³⁰ HSPs
350 including HSP70-1A/B, were reported in dozen studies to be related with meat tenderness in
351 beef^{11, 31} and other species³² from several breeds, animal types and muscles.^{11, 14-15, 26} The Hsp70
352 kDa family proteins are among the most highly conserved protein families found in a wide array
353 of organisms.²⁹⁻³⁰ The role of HSPs in living animals has been extensively described²⁹, however,
354 their action in *post-mortem* muscle is still unclear, although several studies have reported
355 correlations between them and tenderness.¹¹ The stress-related HSP70 protein and molecular
356 chaperone were in this study negatively and strongly related with sensory tenderness and
357 positively with shear force measurements (WBSF). Accordingly, in the majority of proteomic
358 beef tenderness investigations, large HSPs have consistently been reported to differ between
359 tender and tough beef samples and were also found to be correlated in the same directions than
360 in our study with instrumental and sensory tenderness (for review¹¹). The robust link with
361 tenderness, *i.e.*, high accuracy of the models, agrees with the current consensus that HSPs would
362 play in meat tenderness determinism in consequence to the interruption in the supply of nutrients
363 and oxygen as well as to pH drop on triggering cell death by apoptosis.^{6, 8} For example, the

364 negative relationship with sensory tenderness is in line with the findings by Guillemain *et al.*³³ in
365 Charolais cattle of both steers and young bulls. Thus, less abundance of this protein in animal
366 carcasses with lower meat shear force agrees with the anti-apoptotic action that it would play
367 during muscle to meat conversion. Indeed, the strong correlation of tenderness with HSP70-
368 1A/B may be interpreted through three actions: i) a response to cellular stress since this protein
369 has been reported to be induced in skeletal muscle, ii) binding to the structural proteins to
370 maintain homeostasis or iii) protection roles of *post-mortem* muscle cells.^{6, 30} Thereby, it is
371 interesting to establish that in both LT and ST muscles, HSP70-1A/B protein could be considered
372 as a good indicator of beef tenderness of young bulls. It is worthwhile to note HSP70-1 being
373 linked to carcass characteristics², rearing practices², meat color³⁴, water holding capacity³⁵,
374 animal stress³⁶ and to meat tenderness. This suggests that evaluating the abundance of this
375 inducible chaperone could offer a way to predict and to control early *post-mortem* the tenderness
376 potential of carcasses whatever the type of muscle considered. Furthermore, these findings
377 confirm the number of profiling proteomic studies, which have reported the involvement of large
378 HSP proteins including HSP70-8 (retained in this trial only for LT muscle with both tenderness
379 measurements, **Figure 1**) in the determinism of tenderness and other beef sensory qualities.¹²

380 *Dressing percentage a robust carcass predictor with muscle-dependency*

381 Dressing percentage is the second variable (from slaughterhouse level) that was strongly
382 retained in the regression models to explain beef tenderness irrespective of the muscle and the
383 evaluation method (**Figure 1**). The effect of this carcass trait on tenderness was found in this
384 study to be muscle-dependent as it differs in its direction in the regression models between the
385 two muscles, *i.e.*, negative for LT and positive for ST sensory tenderness (the inverse is true for
386 WBSF). Dressing percentage is one of many factors affecting the economic value and production
387 potential of a slaughtered animal. This indicator already described above, is calculated by
388 dividing the hot carcass weight by the live weight of the animal. To our knowledge, there is no
389 study in the large literature that found this robust muscle-dependent relationship to explain
390 tenderness. In agreement with the findings of the present study and in a large dataset of 308
391 young bulls, we reported dressing percentage to be further retained as a good predictor (splitter)
392 using decision trees to categorize meat cuts into tender, medium and tough meat samples
393 classes.¹³ Overall, the findings of this study concerning this carcass trait stress forward the
394 importance of muscle protein turnover and development. For example, muscle protein turnover
395 was reported to vary among muscles, and was reported to be larger in muscles rich in oxidative
396 fibers.³⁷ Protein turnover was further reported to affect the final meat quality including

397 tenderness.³⁸ Indeed, it is worth emphasizing that carcass weight that is closely related to lean
398 carcass percentage,³⁹ was reported to affect meat quality, including tenderness. Overall, in the
399 present study, any increase in the dressing percentage was at the expense of meat tenderness in
400 *Longissimus thoracis* muscle (LT, mixed fast oxido-glycolytic) but beneficial for
401 *Semitendinosus* (ST, mixed fast glycolytic) muscle. These inverse relationships would be partly
402 explained by two points. First, the mechanisms that control the processes of muscle growth and
403 development of each muscle would differ depending on muscle fibers.^{14,38} Second, the muscle
404 characteristics would further affect the ageing rate and thereby tenderness development. Indeed,
405 earlier studies reported that the ageing rate of the myofibrillar structure is higher in the fast-
406 twitch type fibers than in slow-twitch fibers.⁴⁰ This would be also argued by the fact that
407 increased carcass yield is usually associated with more muscle than fat, thereby with high fast
408 glycolytic type fibers.³⁸

409 ***Variables of interest that were retained in three situations***

410 The findings highlighted the interest of HSP20, ENO3, MyHC-I as three muscle protein
411 biomarkers, and dry matter intake (DMI) as the only variable from the farmgate level to be
412 retained at three-quarter of the four possible situations to explain beef tenderness (**Figure 1**).
413 ENO3 and HSP20 were for ST-tenderness, ST-WBSF and LT-tenderness; DMI was the inverse
414 of this former situation as it was retained for LT-tenderness, LT-WBSF and ST-tenderness and
415 the last protein, MyHC-I (in AU), was retained for LT-tenderness, LT-WBSF and ST-WBSF
416 (**Figure 1**). This study is the first to show these kind of links by considering muscle-type and
417 evaluation method of tenderness. These results partly agree to our previous studies depicting
418 muscle specificity in the relationships as well as the type of direction (sign) in the models.^{14-15,}
419 ²⁶ A recent work aiming to predict beef texture estimated by different traits (sensory tenderness,
420 WBSF, residues and juiciness), highlighted the effect of the evaluation of tenderness method on
421 the type of relationships within 29 protein biomarkers as explanatory factors.²⁶

422 In this trial, the four retained variables in three situations of four to explain tenderness
423 variability were already described in the large literature to play an important role in muscle to
424 meat conversion and/or of beef tenderness determinism and prediction. HSP20 is a small HSP
425 (sHSP) that is expressed at high levels in many mammalian tissues including skeletal muscle.⁴¹
426 The involvement of sHSP in the regression models would be explained by the protective role of
427 cytoskeletal proteins^{6, 11, 41} as well as the control of redox status of *post-mortem* muscle cells.⁴²
428 It is also well known that changes in HSP20 levels may occur in response to metabolic activity
429 and pH decline (admitted to affect meat tenderness development), to link with or stabilize

430 myofibrillar proteins.⁴³ In agreement with our findings for the two tenderness measurements,
431 earlier studies reported both positive⁴⁴ and negative^{25-26, 45} relationships between HSP20 and beef
432 tenderness. From these studies, our group have recently validated HSP20 using Reverse Phase
433 Protein Array technique in LT muscle of young Charolais bulls as a robust negative (sensory
434 tenderness and residues) and positive (juiciness and WBSF) biomarker of texture traits.²⁶
435 Moreover, inverse relationships between tenderness and proteins from the sHSP group including
436 HSP20 were already reported to depend on muscle type and breed¹⁴ and rearing practices.²

437 The second protein, ENO3 (EC: 4.2.1.11), is an enzyme catalyzing the conversion of 2-
438 phosphoglycerate to phosphoenolpyruvate in the penultimate step of glycolysis in connection
439 with mitochondria that was described more abundant in striated muscles.^{6, 11} Its involvement in
440 the tenderness regression models is in line with the direction in the models of the above sHSP
441 protein (HSP20) as it was further more abundant in fat animals.⁴⁶ Indeed, ENO3 was described
442 as a hypoxic stress protein implicated in cellular protection during hypoxia⁴⁷. This is in coherence
443 with the high levels of sHSP that may involve cellular stress response under hypoxia or low
444 glucose levels for the muscle cuts.⁴⁸

445 The third retained protein variable was MyHC-I expressed in slow oxidative fibers. This
446 strongly supports the involvement of sHSP. In fact, this is in coherence with earlier reports as
447 sHSP are known to be more abundant in slow oxidative fibers.⁴⁹ The link of this structural protein
448 in meat tenderness was extensively reported in the large literature namely in continental
449 European breeds.^{11-12, 25, 44-45} This was evidenced to be due to proteolysis (high *post-mortem*
450 fragmentation) as revealed by the earlier studies⁵⁰ although the existence of breed, muscle and
451 aging effects.⁵¹

452 The fourth variable, dry matter intake (DMI) belongs to the first level (farmgate) of the
453 continuum. DMI is a rearing factor that is linearly related with the composition of the diet⁵². The
454 results of Gagaoua *et al.* showed that this variable was among those from the farmgate level that
455 strongly discriminated among three tenderness classes (tough, medium and tender ribeye steaks)
456 as well as flavor and juiciness classes of young bulls⁴. Thus, the present study validated DMI as
457 an important indicator of tenderness prediction and clustering of young bulls carcasses. From the
458 literature, DMI was among the good splitters in the tenderness decision tree built using a large
459 list of rearing factors from a dataset of 308 young bulls.¹³ The link and accuracy of DMI in the
460 tenderness models of this study and in other trials, agrees with the importance of considering this
461 rearing factor for both carcass and meat qualities management.⁵³ For example, several trials
462 highlighted that the carcasses are fatter when the DMI of steers and young bulls are increased

463 ⁴⁸ which would explain the link with tenderness as fat content or fat cover of the carcasses is
464 known to impact sensory qualities of meat.⁴ This statement and in agreement with animal feeding
465 effect on beef cuts liking^{3, 54} was strongly supported by the involvement of DMI in the models
466 of both muscles with specific link for sensory tenderness,

467 ***Specific variables for LT or ST muscles irrespective of the evaluation method of tenderness***

468 Six variables were found to be specific irrespective of the evaluation method of tenderness
469 for LT and five for ST muscle (**Figure 1**). For LT, half of the variables were from the
470 slaughterhouse level with three carcass traits (fatness score, fat carcass % and muscle carcass
471 %), two were muscle characteristics (HSP70-8 and MyHC-IIx/b) and only one meat quality trait
472 pH_{3h}. For ST muscle, two variables were rearing factors namely ADG and feed efficiency and
473 the remaining three variables were all structural protein biomarkers (α -actin, MyBP-H and CapZ-
474 β). From these results, it seems that some carcass characteristics such as fatness score and carcass
475 composition appeared to be drivers of LT muscle tenderness but not of ST muscle. In ST muscle,
476 tenderness is more driven by the use of nutrients of the diet. In this muscle, structural proteins
477 seems to be good indicators of tenderness whereas in LT, tenderness is more related to contractile
478 and metabolic characteristics in relation with muscle to fat ratio. These results are coherent with
479 the higher intramuscular fat content of LT comparatively to ST muscle.⁵⁵ These differences
480 revealed on one hand the complexity of the mechanisms that underlie muscle to meat conversion
481 and thus tenderness development; and on the second hand the involvement of factors at multiple
482 levels, but in a muscle-dependent direction manner.

483 The hypothesis that among carcass characteristics some of them would have an impact on
484 tenderness was very strongly supported by our findings, namely for LT muscle. This agrees with
485 previous studies that investigated tenderness variability of young bulls.^{4, 19} For example, in
486 previous studies marbling was found to account in the variation of aged beef tenderness.⁵⁶
487 Similarly, average daily gain and feed efficiency from the farmgate level were further reported
488 to drive the variability in meat quality of cattle.⁵⁷ From these two variables, feed efficiency was
489 suggested as a prerequisite in the beef industry for tenderness management⁵⁴ as it can contribute
490 to differences in protein turnover and tissue metabolism among animals with effects on some
491 proteolytic systems such as calpains.⁵⁸ It is well known from large literature that the calpain
492 system plays an important role both in *post-mortem* tenderization⁶ and *in vivo* muscle growth⁵⁹,
493 and it is further associated with meat toughness as well as lean growth and feed efficiency in
494 cattle.³⁸ These would partly explain the involvement in ST muscle of three muscle and structural
495 proteins with strong association to tenderness variability.^{11-12, 26} In agreement with our findings,

496 previous proteomic studies identified α -actin^{2, 11, 44-45}, MyBP-H^{25-26, 60} and CapZ- β ⁶¹ as
497 biomarkers of beef tenderness. Meat tenderness is achieved primarily through ageing, during
498 which structural changes occurring in the muscle lead to complex mechanisms involving pH and
499 ionic strength, combined with the action of cellular proteolytic systems.⁶ From these proteins, α -
500 actin was extensively identified in proteomic studies¹¹⁻¹² and, postulated by our group to play a
501 pivotal role as a hallmark of apoptosis onset.⁶ In addition, MyBP-H has significant effects on
502 length, thickness, and lateral alignment of myosin filaments⁶², which explain its involvement
503 together with other structural proteins as well as the rearing factors reported to play a role in
504 muscle growth and metabolism. Located in the A-band of the myofibrils, MyBP-H is closely
505 related with the thick filaments explaining its implication in the determinism of beef tenderness.
506 Furthermore, we have recently found and suggested the pivotal role that MyBP-H would play in
507 muscularity development under a marked rearing practice effect.²

508 On another hand, pH_{3h} was found to be retained in the explanatory models of LT muscle only
509 whatever the evaluation method (negatively with sensory tenderness and positively with WBSF).
510 This is consistent with the fast oxidative nature of LT comparatively to ST muscle. It has been
511 suggested in the earlier studies that the pH at 3 h would be a valuable indicator of tenderness.⁶³⁻
512 ⁶⁴ The involvement of pH, agrees with the numerous previous studies reporting the effect of rate
513 and extent of pH decline of muscle, on meat conversion.^{6, 34, 40} For example, the study of
514 Dransfield *et al.* reported that the relationship of overall tenderness to pH at 3 h was linear with
515 pH and accounting for 0.52 of the variation in tenderness among age groups and muscle types of
516 French cull cows and young bulls.⁶⁴

517 Overall, the findings of this part highlight that the variables or factors that would be used for
518 tenderness explanatory models irrespective of the evaluation method, are partly muscle
519 dependent and need to be considered in the future investigations to develop accurate tools or to
520 improve the existing tools for muscle cuts evaluation for their tenderness potential.

521 ***Common variables for LT and ST muscles irrespective of tenderness evaluation method***

522 For WBSF only and irrespective of muscle, LDH-B was retained positively in the explanation
523 model of LT and negatively for ST muscle. LDH-B is a glycolytic enzyme that catalyzes the
524 inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD⁺.
525 The findings of the present study agree with previous trials by our group reported using
526 regression analyses¹⁴ or meta-analysis.⁹ In the study by Chriki *et al.*⁹ and in line to our findings,
527 the LDH activity was also positively correlated with sensory tenderness in the ST, of cows

528 whereas it was negatively correlated with WBSF in young bulls. Moreover, LDH-B was already
529 identified by proteomics as a potential biomarker of meat tenderness whatever the evaluation
530 method.^{11-12, 14-15}

531 This study showed that other variables were specific to LT or ST muscles and for each
532 tenderness trait (**Figure 1**). This highlights the difficulty of developing indicators or biomarkers
533 for tenderness prediction without considering the specificities that exist among muscles and
534 tenderness evaluation methods. Meat tenderizing known as a finely orchestrated process is
535 strongly comforted by the findings of this study using two evaluation methods of beef tenderness
536 and two divergent muscles. The study highlighted further the existence of tenderness drivers at
537 different levels of the continuum from farmgate-to-meat by providing the positive and negative
538 impacts. From this study, it appears that HSP70-1A/B could be a relatively generic biomarker of
539 tenderness whatever the evaluation method and muscle.

540 Overall, this trial highlighted the importance of studying the effects of multiple combined
541 factors on beef tenderness rather than simply evaluating the links within each level or factor
542 separately. We propose the implementation of this approach to explore accurately meta-data that
543 come from several experiments grouping thousand animals, to provide decision tools for beef
544 sector seeking to manage beef tenderness variability. This is strongly supported by the
545 consistencies and differences that were revealed by the explanatory models of this study. Based
546 on the rank of the variables in the models (**Tables 4 and 5**) as well as the results shown in the
547 Venn diagram (**Figure 1**), it seems that tenderness for LT muscle is mainly affected by carcass
548 characteristics (dressing %, fatness score and fat to muscle ratio). However, for ST muscle,
549 tenderness is mainly dependent of factors related to the fattening period such as ADG and feed
550 efficiency including DMI.

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Figure captions

Figure 1. Venn diagrams depicting a summary from the **Tables 4 and 5 of A)** the retained variables in the PLS models for the two muscles (*Longissimus thoracis* (LT) vs. *Semitendinosus* (ST)) and beef tenderness attributes (assessed by a sensory panel vs. instrumental measurement (WBSF)); and **B)** the number of variables retained or shared in each set within muscles-tenderness traits. For further details concerning the abbreviations and direction of the variables in the models, refer to **Tables 4 and 5.**

Table 1. Descriptive statistics of the 16 variables corresponding to data from farmgate level (animal characteristics and rearing factors) and slaughterhouse level (carcass characteristics) measured for the studied young bulls.

	Mean	SD	Min	Max
<i>Farmgate level: animal characteristics and rearing factors (q = 8)</i>				
Age at slaughter, month	16.6	1.0	14.0	18.8
Initial body weight, kg	498.9	32.4	439.2	590.8
Slaughter body weight, kg	632.0	47.5	497.0	745.0
Forage, %	24.2	4.2	8.8	31.2
Concentrate, %	75.7	4.1	68.8	90.4
Dry matter intake (DMI), kg DM/day	10.4	1.5	7.5	13.7
BW gain (average daily gain-ADG), kg/d	1.43	0.28	0.52	2.12
Feed efficiency (ADG:DMI ratio), kg/kg DM	138.5	26.5	56.9	213.1
<i>Slaughterhouse level: carcass characteristics (q = 8)</i>				
Empty body weight, kg	573.8	44.0	443.8	686.7
Cold carcass weight, kg	394.7	36.6	285.0	500.4
6 th rib weight, kg	2.19	0.27	1.72	3.47
%Fat carcass ¹	14.5	4.3	7.9	25.8
%Muscle carcass ¹	72.0	4.6	61.4	78.5
Dressing, %	60.9	4.9	42.7	68.0
Conformation score, 1 – 15 scale ²	9.8	1.3	7.0	12.0
Fatness score, 1 – 15 scale ³	6.6	2.3	2.0	11.0

¹ Muscle and fat carcass weights were estimated from the 6th rib composition after dissection as described by Gagaoua *et al.*¹⁹. Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

² EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

³ EUROP classification grid for carcass fatness scores from 1 = leanest to 15 = fattest.

Table 2. Descriptive statistics of the 35 variables corresponding to data from muscle level (muscle characteristics and protein biomarkers) and meat level (meat quality traits) measured for the studied young bulls in *Longissimus thoracis* muscle.

	Mean	SD	Min	Max
Muscle level: muscle characteristics and protein biomarkers (q = 27)				
<i>a. Contractile properties by myosin fibers characterization</i>				
Myosin heavy chain-I, % (MyHC-I)	23.50	6.18	12.30	42.30
Myosin heavy chain-IIa, % (MyHC-IIa)	35.31	13.79	16.50	63.90
Myosin heavy chain-IIx/b, % (MyHC-IIx/b)	41.21	17.81	1.70	64.70
<i>b. Intramuscular connective tissue properties</i>				
Total collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	3.62	0.85	2.54	6.65
Insoluble collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	2.74	0.69	1.83	4.97
Soluble collagen, %	24.10	10.00	5.50	53.80
<i>c. Protein biomarkers quantified by Dot-Blot (in arbitrary units)</i>				
Heat shock proteins				
$\alpha\text{B-crystallin}$	20.01	7.05	9.82	46.68
HSP20	18.46	4.26	11.34	36.86
HSP27	22.70	9.09	8.39	55.95
HSP40	17.04	2.21	12.14	22.09
HSP70-8	16.68	1.81	13.11	21.79
HSP70-1A/B	18.28	3.85	11.30	28.79
HSP70-Grp75	12.76	4.33	6.95	20.47
Metabolism				
Enolase 3 (ENO3)	15.14	4.73	7.30	32.67
Lactate dehydrogenase chain B (LDH-B)	14.73	4.98	5.87	27.46
Malate dehydrogenase 1 (MDH1)	14.07	3.78	7.01	23.14
Structure				
F-actin-capping protein subunit β (CapZ- β)	16.77	3.49	10.64	27.60
α -actin	17.62	3.38	11.20	26.83
Myosin light chain 1F (MyLC-1F)	14.94	1.67	10.76	18.18
Myosin binding protein H (MyBP-H)	14.02	7.11	4.72	41.08
Myosin heavy chain-I (MyHC-I)	17.70	2.79	11.70	24.35
MyHC-II	15.14	2.54	10.22	21.26
MyHC-IIx	14.50	9.09	2.28	31.39
Oxidative stress				
Superoxide dismutase [Cu-Zn] (SOD1)	16.27	3.49	10.62	40.54
Peroxiredoxin 6 (PRDX6)	14.00	1.87	9.71	18.50
Protein deglycase (DJ1)	16.55	2.79	11.58	26.05
Proteolysis				
μ -calpain	14.76	2.36	6.91	20.46
Meat level: meat quality traits (q = 8)				
Sensory tenderness, 0 – 10 scale	4.94	0.72	3.17	6.31
Warner-Bratzler shear force (N/cm ²)	42.27	10.51	27.34	78.31
pH _{45min}	6.88	0.12	6.52	7.15
pH _{3h}	6.18	0.28	5.52	6.84
pHu	5.65	0.14	5.31	6.13
Lightness (L*)	36.25	3.25	26.07	43.20
Redness (a*)	13.47	2.26	9.34	20.87
Yellowness (b*)	14.53	3.21	8.77	21.93

Table 3. Description of the list of the 32 variables corresponding to data from muscle level (muscle characteristics and protein biomarkers) and meat level (meat quality traits) measured for the studied young bulls in *Semitendinosus* muscle.

	Mean	SD	Min	Max
Muscle level: muscle characteristics and protein biomarkers (q = 27)				
<i>a. Contractile properties by myosin fiber characterization</i>				
Myosin heavy chain-I, % (MyHC-I)	7.47	4.56	1.10	30.90
Myosin heavy chain-IIa, % (MyHC-IIa)	24.01	7.34	12.30	45.00
Myosin heavy chain-IIx/b, % (MyHC-IIx/b)	68.53	10.83	27.00	84.30
<i>b. Intramuscular connective tissue properties</i>				
Total collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	5.33	0.96	3.86	8.16
Insoluble collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	3.75	0.74	2.21	5.26
Soluble collagen, %	29.38	10.67	2.36	50.34
<i>c. Protein biomarkers quantified by Dot-Blot (in arbitrary units)</i>				
Heat shock proteins				
$\alpha\text{B-crystallin}$	60.05	25.39	21.06	186.27
HSP20	87.94	19.45	53.15	139.85
HSP27	107.17	33.00	51.09	228.07
HSP40	87.06	11.94	54.12	119.39
HSP70-8	104.29	10.46	84.72	139.32
HSP70-1A/B	83.67	16.32	54.24	138.55
HSP70-Grp75	84.01	13.50	59.90	113.65
Metabolism				
Enolase 3 (ENO3)	90.05	19.68	57.52	140.26
Lactate dehydrogenase chain B (LDH-B)	89.29	18.38	48.72	133.88
Malate dehydrogenase 1 (MDH1)	99.39	20.32	63.71	166.43
Structure				
F-actin-capping protein subunit β (CapZ- β)	93.23	15.58	53.64	141.36
α -actin	117.07	17.34	69.37	154.52
Myosin light chain 1F (MyLC-1F)	101.60	9.74	74.32	135.95
Myosin binding protein H (MyBP-H)	84.51	19.69	42.97	133.13
Myosin heavy chain-I (MyHC-I)	81.11	19.39	37.28	133.37
MyHC-II	112.05	12.51	84.28	137.34
MyHC-IIx	160.45	42.93	50.42	264.10
Oxidative stress				
Superoxide dismutase [Cu-Zn] (SOD1)	111.17	66.27	73.11	650.10
Peroxiredoxin 6 (PRDX6)	107.49	12.81	82.26	134.15
Protein deglycase (DJ1)	90.14	14.17	64.05	152.31
Proteolysis				
μ -calpain	95.95	14.43	67.75	131.03
Meat level: meat quality traits (q = 5)				
Sensory tenderness, 0 – 10 scale	4.59	0.44	3.64	5.67
Warner-Bratzler shear force (N/cm ²)	44.67	8.43	28.86	73.20
pH _{45min}	6.67	0.12	6.28	6.98
pH _{3h}	5.91	0.27	5.35	6.45
pHu	5.56	0.14	5.37	6.07

Table 4. Ranking of the retained variables, according to their VIP, of the second PLS meat sensory tenderness model in *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of young bulls. The regression coefficients (β) from PCR models of the variables belonging to each level of the continuum are given including the standard errors (SE)¹.

Continuum data (LT muscle)	PLS (R ² = 56%)		PCR (R ² = 68%)		Continuum data (ST muscle)	PLS (R ² = 52%)		PCR (R ² = 59%)	
	Rank	VIP	β	SE		Rank	VIP	β	SE
<i>Farmgate level: animal characteristics and rearing factors</i>									
DMI, kg DM/day	1	2.18	+0.53	0.13	ADG, kg/d	1	2.97	-0.73	0.93
					Feed efficiency, kg/kg DM	2	2.46	+0.81	0.78
					DMI, kg DM/day	10	1.19	+0.56	0.45
<i>Slaughterhouse level: carcass characteristics</i>									
Fatness score, 1 – 15 scale	4	1.64	+0.12	0.17	Dressing, %	11	1.09	+0.28	0.13
Fat carcass, %	9	1.46	+0.37	0.57					
Muscle carcass, %	10	1.41	-0.82	0.60					
Dressing, %	13	1.10	-0.21	0.14					
<i>Muscle level: muscle characteristics and protein biomarkers</i>									
ENO3, arbitrary units (AU)	2	1.86	+0.47	0.18	HSP70-1A/B, AU	3	2.01	-0.22	0.13
MyHC-IIa, %	3	1.69	+0.47	0.22	SOD1, AU	4	1.70	-0.05	0.12
HSP70-8, AU	5	1.61	-0.21	0.20	α -actin, AU	5	1.51	-0.07	0.12
HSP27, AU	6	1.60	-0.21	0.14	MyBP-H, AU	6	1.46	+0.08	0.14
HSP70-1A/B, AU	7	1.59	-0.61	0.20	MLC-1F, AU	7	1.41	+0.12	0.13
HSP20, AU	8	1.52	+0.50	0.15	ENO3, AU	8	1.38	-0.03	0.12
MyHC-IIx/b, %	11	1.35	+0.49	0.22	HSP20, AU	9	1.37	-0.05	0.12
MyHC-I, AU	14	1.10	-0.24	0.18	CapZ- β , AU	12	1.01	+0.02	0.04
Soluble collagen, %	15	1.04	-0.07	0.13					
PRDX6, AU	16	1.00	+0.14	0.15					
<i>Meat level: meat quality traits</i>									
pH _{3h}	12	1.11	-0.09	0.13					

¹ The variables from the different levels of the continuum that are in bold character highlight those retained in the models of both muscles; and those in bold and italic character share the same direction (- or +) in the PCR models of both muscles.

Table 5. Ranking of the retained variables, according to their VIP, of the second PLS Warner-Bratzler Shear Force (WBSF) model in *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles of young bulls. The regression coefficients (β) from PCR models of the variables belonging to each level of the continuum are given including the standard errors (SE)¹.

Continuum data (LT muscle)	PLS (R ² = 57%)		PCR (R ² = 66%)		Continuum data (ST muscle)	PLS (R ² = 53%)		PCR (R ² = 63%)	
	Rank	VIP	β	SE		Rank	VIP	β	SE
<i>Farmgate level: animal characteristics and rearing factors</i>									
Age at slaughter, months	1	2.22	-0.23	0.14	Initial BW, kg	1	2.36	+0.28	0.14
Concentrate, %	2	2.22	+0.62	0.26	ADG, kg/d	12	1.21	+0.30	0.26
Forage, %	3	2.20	+0.40	0.29	Feed efficiency, kg/kg DM	13	1.02	-0.33	0.25
DMI, kg DM/day	10	1.20	-0.20	0.12					
<i>Slaughterhouse level: carcass characteristics</i>									
Fatness score, 1 – 15 scale	8	1.33	-0.05	0.08	Dressing, %	8	1.39	-0.02	0.13
Muscle carcass, %	11	1.18	+0.76	0.59					
Fat carcass, %	12	1.08	-0.89	0.57					
Dressing, %	14	1.00	+0.08	0.14					
<i>Muscle level: muscle characteristics and protein biomarkers</i>									
HSP70-8, arbitrary units (AU)	5	1.88	+0.10	0.18	CapZ- β , AU	2	1.93	-0.10	0.13
<i>HSP70-1A/B, AU</i>	6	1.80	+0.16	0.19	<i>HSP70-1A/B, AU</i>	3	1.87	+0.14	0.13
<i>MyHC-I, AU</i>	7	1.35	+0.03	0.16	<i>MyHC-I, AU</i>	4	1.85	+0.05	0.14
LDH-B, AU	9	1.26	+0.22	0.13	LDH-B, AU	5	1.82	-0.30	0.13
MyHC-IIx/b, %	13	1.05	-0.04	0.13	HSP20, AU	6	1.69	+0.22	0.13
					ENO3, AU	7	1.68	+0.21	0.15
					MyHC-II, AU	9	1.37	+0.09	0.13
					MDH1, AU	10	1.35	+0.01	0.07
					α -actin, AU	11	1.27	+0.21	0.12
					MyBP-H, AU	14	1.01	-0.11	0.13
<i>Meat level: meat quality traits</i>									
pH _{45min}	4	1.93	+0.24	0.13					
pH _{3h}	15	1.00	+0.12	0.12					

¹ The variables from the different levels of the continuum that are in bold character highlight those retained in the models of both muscles; and those in bold and italic character share the same positive direction in the PCR models of both muscles.

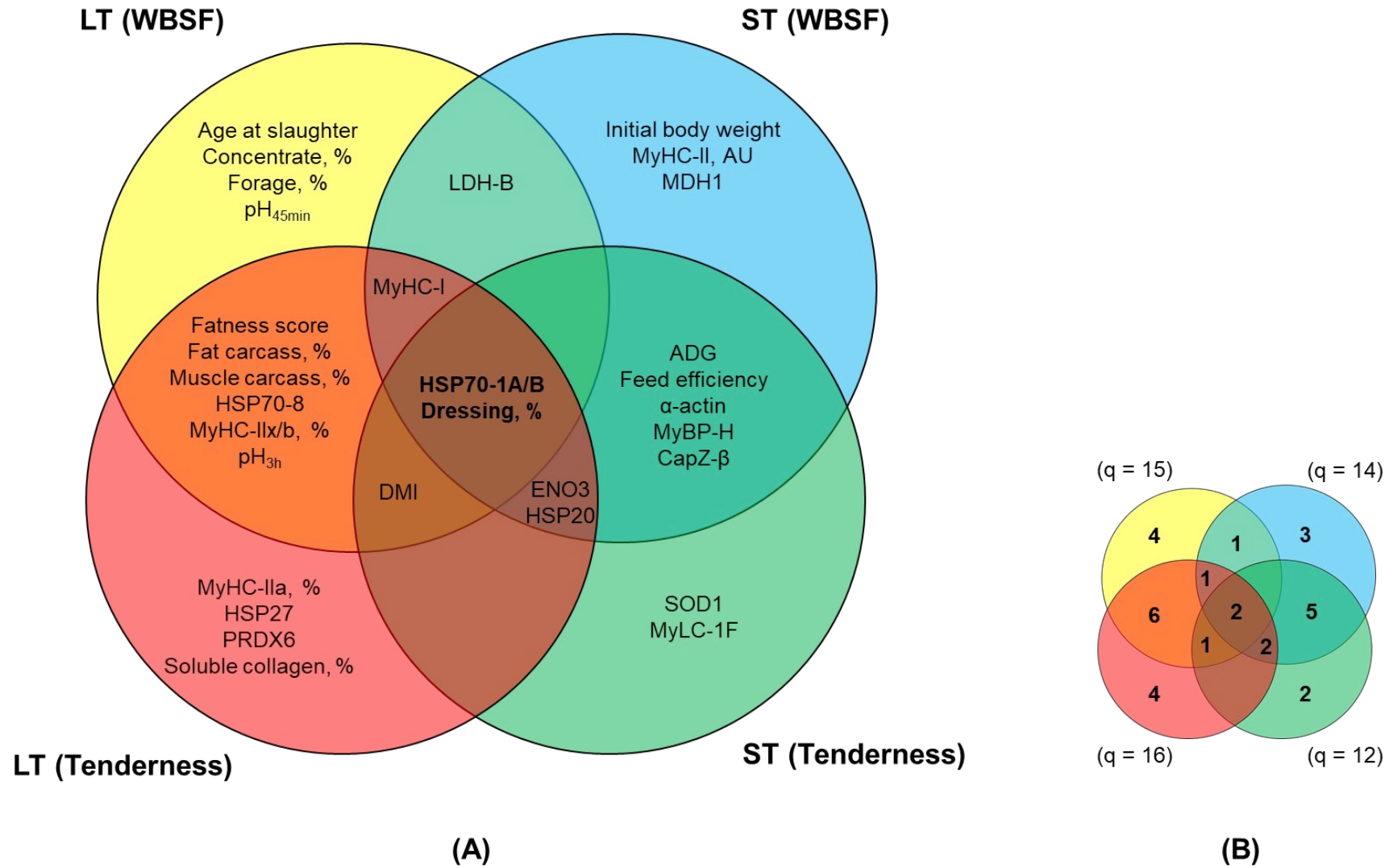


Figure 1.

TOC

