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3 1 **Assessment of cattle inter-individual cluster variability: the potential of continuum data**
4 2 **from the farmgate-to-fork for ultimate beef tenderness management**

5 3
6 4
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28 15 **Abstract:**

29 16 **Background:** This study implemented a holistic approach based on the farmgate-to-fork data
30 17 at the 4 levels of the continuum (farmgate – slaughterhouse – muscle – meat) to study the inter-
31 18 individual cluster variability of beef tenderness. For that, 171 young bulls were selected on a
32 19 large database of 480 animals according to the industrial expectations based on animal and
33 20 carcass characteristics. The targeted factors were age at slaughter [14; 20 months], carcass
34 21 weight [370; 470 kg], EUROP conformation [7; 15] and fatness [2.5; 5] scores of the carcasses.
35 22 Multivariate analyses and unsupervised learning tools were performed.

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39 23 **Results:** Principal component analysis combined to agglomerative hierarchical clustering
40 24 allowed to identify 10 clusters that differed ($P < 0.0001$) for the 4 targeted factors. The clusters
41 25 were further different for variables belonging to each level of the continuum. The results
42 26 indicated an inter-individual cluster variability rising in tenderness in link with the continuum
43 27 data grouped according to industrial expectations. The associations of the whole variables of
44 28 the continuum with tenderness were both linear and curvilinear and some of them, such as
45 29 fatness scores, fat carcass weight and dressing per cent were best described by quadratic Bezier
46 30 curves. The findings showed that the variables contributing most to the inter-individual cluster
47 31 variability of tenderness seemed to be more related to the rearing practices, mainly feeding, and
48 32 their consequences on carcass properties than to muscle characteristics.

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53 33 **Conclusion:** It seems that considering the continuum data would allow possible trade-off
54 34 managements of tenderness to identify levers at different levels from the farmgate-to-meat.

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58 37 **Key words:** Beef tenderness; Young bulls; Carcass and muscle characteristics; Rearing factors;
59 38 Multivariate analyses.

39 INTRODUCTION

40 Tenderness was reported by numerous studies as the most important beef palatability trait¹
41 for the consumers, with evidence demonstrating that they are willing to pay a premium for
42 steaks that are guaranteed tender². More recent studies have further shown that when tenderness
43 reaches an acceptable level, flavor and juiciness become very important drivers of beef eating
44 satisfaction³. These constitute internationally the major beef sensory quality traits to be
45 controlled by beef industry for consumers' satisfaction. However, in most European countries,
46 the current EUROP grading system does not include meat quality traits such as tenderness in
47 the carcasses evaluation at the slaughterhouse⁴. Thus, carcasses are not priced on the basis of
48 their tenderness potential (or other qualities), so producers lack incentive to guaranty tender
49 beef. As a result, consumer expectations are not fully considered. That is why beef producers,
50 are seeking for accurate tools that would allow predicting or categorizing carcasses for their
51 tenderness potential as well as other sensory qualities soon after animal bleeding.

52 However, to develop predictive decision tools, several factors at different levels from the
53 farmgate-to-fork, known as factors of the continuum, contribute to the variability of the eating
54 qualities⁵⁻⁷ and thus of consumer satisfaction^{8, 9}. Indeed, the farm management systems and
55 rearing factors, especially during the fattening period, were reported to contribute to the
56 variation of both the final carcass and meat quality traits^{5, 10, 11}. These variations are likely to be
57 due to various intrinsic and extrinsic factors such as breed, sex, age, feeding, rearing factors,
58 transport and animal handling, muscle type by metabolic and contractile properties, post-
59 slaughter ageing and many others¹²⁻¹⁵, which interact and determine the outcome of metabolic
60 processes in the *peri*- and *post-mortem* period and meat tenderness. Furthermore, previous
61 studies reported that there is a large individual variation in beef tenderness within animals¹⁶ and
62 also between animals with similar breed, sex, age and fattening system. Earlier studies at the
63 breed or muscle level have shown that the genetic effect alone can't explain the observed
64 differences in carcass and meat traits because the genetic control was weak within breeds^{16 17}.
65 In this context, the main objective of the beef sector is a better understanding of the variability
66 existing between or within individuals. We hypothesized that this would be possible if we
67 provide more evidence about the impact of inter-individual variability of the animals on carcass
68 and meat quality traits. Implementation of a holistic approach by considering the farmgate-to-
69 fork data at the 4 levels of the continuum (farmgate – slaughterhouse – muscle – meat) of the
70 animals was relevant to characterize sufficiently those factors in relation to the desirable beef
71 qualities. This would further allow better understanding of the inter-individual variability

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3 72 within animals produced under similar conditions or that share similar characteristics. Thus,
4 73 this study on a homogeneous dataset of 171 French young bulls from a large INRA database
5 74 aimed to investigate tenderness variability among homogenous animal population chosen
6 75 according to the industrial expectations based on animal and carcass characteristics considered
7 76 in the EUROP grid system. In this study, we performed multivariate analyses and unsupervised
8 77 learning tools to approach the animal variability of individuals in link with the continuum data.

13 78 **MATERIALS AND METHODS**

15 79 *Animals handling*

16 80 This study was carried out as part of a research program conducted in the context of
17 81 suitability of French beef market to respond to the queries identified by the beef sector namely
18 82 those of the 6 biggest slaughterers of the Auvergne-Rhône-Alpes (ARA) region, France. Thus,
19 83 from an INRA-ARA database of 480 young bulls⁵, 171 animals of 3 breeds *i.e.*, 124 Charolais,
20 84 24 Limousin and 23 Salers were extracted. Data extraction was based on 4 factors that were
21 85 defined thanks to a field survey within industrials (butchers) conducted to identify and to fix
22 86 the range limits of the desirable animal and carcass characteristics. The 4 factors and their fixed
23 87 ranges were age at slaughter [14; 20 months], carcass weight [370; 470 kg], conformation score
24 88 [7; 15, on a 1–15 scale from the EUROP grid] and fatness score [2.5; 5, on a 1–5 scale from the
25 89 EUROP grid] of the carcasses.

26 90 The respondents (managers of the slaughterhouses) were face-to-face interviewed using a
27 91 written semi-structured questionnaire consisting of simple open-ended questions for 29 items
28 92 including the 4 considered criteria in this study.

29 93 The 171 young bulls belong to the major suckled breeds slaughtered in France. The dataset
30 94 belong to previous animal studies conducted according to procedures approved by the regional
31 95 ethical committee and in accordance with applicable French and European guidelines.⁵ The data
32 96 were of no breed effect concerning the 4 criteria factors (**Table S1**). The animals were raised
33 97 and managed under similar experimental conditions in the INRA research center.^{5, 11} They had,
34 98 *ad libitum*, the similar feeding but at different proportions consisting of forages (including
35 99 straw, hay, grass silage, corn silage, and beet pulp silage) and concentrate (containing
36 100 dehydrated alfalfa, grain corn, soybean, urea, wheat, and rapeseed).

37 101 *Animal characteristics and rearing factors*

38 102 Eight rearing factors were collected during the fattening period (**Table 1**) as described by
39 103 Gagaoua *et al.*⁵. They included slaughter age (months), initial body weight in kg at the

1
2
3 104 beginning of the fattening period, slaughter body weight (kg), forage and concentrate in per
4
5 105 cent, fattening duration (days), dry matter intake (DMI, kg DM/day) and energy intake
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7 106 (Mcal/day). Means values, standard deviation and ranges of each factor are given in **Table 1**.

8 9 107 ***Carcass characteristics and composition***

10
11 108 Before slaughter, all animals were fasted for 24 h and had free access to water. They were
12
13 109 stunned using a penetrative captive bolt, prior to exsanguination. Slaughtering was performed
14
15 110 in compliance with the French welfare regulations set at the level of European Directive
16
17 111 (2001/88/EC) in the experimental slaughterhouse of INRA Research Center to standardize
18
19 112 slaughtering, chilling and storing procedures. The carcasses were not electrically stimulated
20
21 113 and they were stored between 2 and 4°C until 24 h *post-mortem*.

22 114 Six parameters characterized the carcasses and their composition. First they were graded
23
24 115 under the EU beef carcass classification scheme using experts familiar with the EUROP grid
25
26 116 (Commission Regulation (EC) 1249/2008). Five conformation classes were defined,
27
28 117 represented by the letters E, U, R, O, and P. The scoring consists of a visual assessment of
29
30 118 carcass muscling where carcasses graded as 'E' have the most muscularity, and this decreases
31
32 119 through to 'P' which have the least muscularity. European Union regulations allow for 3
33
34 120 subdivisions of each conformation class, high: "+", medium: "=" and low: "-". Hence, an
35
36 121 incremental scale ranging from 1 to 15 was used, where 1 corresponds to P- (very low muscle
37
38 122 development) and 15 to E+ (very high muscle development)¹⁸. At the same time, the fatness
39
40 123 score of the carcasses, which describes the amount of fat on the outside of the carcass was
41
42 124 numerically scored from 1 = leanest to 5 = fattest. The carcass weight was measured within 2h
43
44 125 of slaughter and was expressed as cold carcass weight, which is 0.98 times the hot carcass
45
46 126 weight. The carcass weight was used to compute the dressing per cent and carcass composition
47
48 127 by muscle and fat carcass weights (**Table 1**). This was estimated from the composition of the
49
50 128 6th rib determined after physical dissection following standard commercial practice involving a
51
52 129 close trimming and deboning¹⁸.

50 130 ***Muscle characteristics by enzyme activities and connective tissue properties***

51
52 131 Muscle samples of *Longissimus thoracis* (LT, mixed fast oxido-glycolytic muscle) were
53
54 132 excised from the right-hand side of the carcass of each animal 45 min and 24 h *post-mortem*
55
56 133 and 3 parts were distinguished. The first part of the samples taken at 45 min *post-mortem* was
57
58 134 subsequently frozen in liquid nitrogen and kept at -80 °C until analyzed for metabolic enzymes
59
60 135 activities. The second part was cut into pieces of 1–2 cm cross-section, vacuum packed, and

1
2
3 136 stored at -20°C until analyzed for intramuscular fat and collagen contents. The last part was
4
5 137 taken at 24 h *post-mortem*. The epimysium was carefully dissected and the samples were cut
6
7 138 into 2 cm thick steaks and placed in sealed plastic bags under vacuum and kept at 4°C for ageing
8
9 139 (14 days). Each ribeye steak was then frozen and stored at -20°C waiting sensory evaluation.

10
11 140 The metabolic muscle type was determined by measuring glycolytic and oxidative enzyme
12
13 141 activities of respectively, lactate dehydrogenase (LDH; EC 1.1.1.27) and isocitrate
14
15 142 dehydrogenase (ICDH; EC 1.1.1.42)¹⁴. These enzymes are representative of different steps of
16
17 143 energy metabolism used to characterize the metabolic types of beef muscles.

18 144 The intramuscular connective tissue properties were determined from frozen muscle. The
19
20 145 samples were homogenized in a household cutter, freeze-dried for 48 h and pulverized in a
21
22 146 horizontal blade mill. For total collagen, about 250mg of muscle powder were weighed, acid
23
24 147 hydrolyzed with 10mL of 6 N HCl, overnight at 110°C in a screw-capped glass tube. Then, the
25
26 148 acid hydrolysate was diluted 5 times in 6 N HCl and the subsequent procedure was as described
27
28 149 by Dubost *et al.*¹⁹ For insoluble collagen, muscle powder was solubilized according to the
29
30 150 method of Hill²⁰ and hydrolyzed according to the same method¹⁴. For both total and insoluble
31
32 151 collagen, each sample was weighed and measured in triplicate and data were expressed in mg
33
34 152 of hydroxyproline per g of dry matter.

34 153 ***Sensory and technological/chemical meat quality traits***

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36 154 The ultimate pH of the carcasses (ribeye steaks) were evaluated at 24 h *post-mortem*. The
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38 155 measurements were conducted by inserting a glass electrode on five different locations per steak
39
40 156 using a Hanna HI 9025 pH/ORP meter suitable for meat penetration (Hanna Instruments Inc.,
41
42 157 Woonsocket, RI, USA)¹⁸. The pH meter was calibrated at chilling temperature using pH-4 and
43
44 158 pH-7 buffers.

45
46 159 The intramuscular fat (IMF), was evaluated by mixing 6g of lyophilized muscle powder with
47
48 160 chloroform-methanol for extraction before assaying gravimetrically²¹. A Dionex ASE 200
49
50 161 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA) was used. In total, the
51
52 162 meat powder was placed in a 22mL extraction cell previously prepared with a cellulose filter
53
54 163 and silicon balls. The extraction was performed at a temperature of 125°C and a pressure of
55
56 164 103bar. The extract, containing fat content and petroleum ether, was collected and transferred
57
58 165 in an evaporation vial previously weighed. After 15 min of evaporation, the vial was placed 17h
59
60 166 in a drying oven at 105°C and then weighed to determine the IMF content in the meat sample.

1
2
3 167 Each sample was measured in triplicate and muscle total lipids were reported as g of fatty acid
4
5 168 per 100 g wet tissue.

6
7 169 The sensory evaluation of the ribeye steaks was performed following the protocol described
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9 170 by Gagaoua *et al.*^{6,22}. Briefly, the frozen steaks were thawed without stacking or overlapping,
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11 171 at 5°C in vacuum packs for 48 h before cooking and sensory assessment at 55°C. One hour
12
13 172 before sensory evaluation, the steaks were cut into approximately 1.50cm thick and grilled on
14
15 173 a double grooved plate griddle (SOFRACA, Morangis, France) heated to 300°C for 30min
16
17 174 before cooking. Steaks were heated for 2min until the end-point temperature of 55 °C in the
18
19 175 geometric center of the steak was reached (measured using a temperature probe (Type K,
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21 176 HANNA HI 98704, Newark)). After grilling, each steak was cut into 20 mm cubes that were
22
23 177 immediately served to 12 panelists²². The panelists rated the steaks on a 10 cm unstructured
24
25 178 line scale or the following sensory attributes: global tenderness (0, extremely tough; 10,
26
27 179 extremely tender), juiciness (0, extremely dry; 10, extremely juicy), and beef flavor intensity
28
29 180 (0, extremely weak; 10, extremely strong) as in Gagaoua *et al.*^{5,22}. The sensory analyses were
30
31 181 performed in a room equipped with individual booths under artificial red light to reduce the
32
33 182 influence of the appearance of the samples. Each tasting booth was equipped with computer
34
35 183 terminals linked to a fileserver running a sensory software program (Fizz v 2.20 h, Biosystemes,
36
37 184 Couternon, France) that facilitated the direct entry of assessor ratings.

35 185 *Statistical analyses*

36
37 186 A total of 23 variables including tenderness were used in this integrative study by
38
39 187 considering the 4 levels of the continuum from farmagte-to-fork (**Tables 1** and **2**). The SAS
40
41 188 statistical software (SAS 9.3, SAS Institute INC, Cary, NC, USA) and XLSTAT 2018.2
42
43 189 (AddinSoft, Paris, France) were used for data analyses. Normal distribution and homogeneity
44
45 190 of the dataset was first tested by the Shapiro-Wilk test ($P>0.05$). Then, a general description
46
47 191 analysis was realized by computing means, standard deviations, minimum and maximum
48
49 192 ranges (**Tables 1** and **2**).

50 193 To study the inter-individual variability among the 171 animals, the 4 factors *i.e.*, age at
51
52 194 slaughter, carcass weight, conformation and fatness scores of the carcasses were centered and
53
54 195 scaled by computing z-scores using PROC STANDARD of SAS. After that, principal
55
56 196 component analyses (PCA) combined with agglomerative hierarchical clustering (HCA) or *k*-
57
58 197 means cluster analyses were undertaken to create animal groups⁶. Two factors with eigenvalues
59
60 198 >1.0 were extracted on the factor loading matrix after orthogonal rotation. The HCA gave the
199 199 best results based on the ranking of the animals according to their similarities and allowed to

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2
3 200 identify 10 animal clusters with less than 5% variation among them for the 4 used factors.
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5 201 Results of HCA are represented graphically by a dendrogram that provides a simple way of
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7 202 visualizing the hierarchical structure of the clustering and the level at which each cluster is
8
9 203 formed, as well as cluster membership. The 10 animal clusters were further confirmed by
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11 204 multidimensional scaling (MDS) dissimilarity, an unsupervised procedure, based on the
12
13 205 Euclidean distances matrix of the dissimilarities that were generated thanks to Young-
14
15 206 Householder theorem²³. The 10 clusters were compared using PROC GLM procedure of SAS
16
17 207 for the whole variables of the continuum after the projection on a new PCA to perform a
18
19 208 between-animal cluster analysis. Therefore, we used both simple linear regression and random
20
21 209 regression methods to model systematic changes in tenderness within clusters by considering
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23 210 homogeneity of variance across individuals. To take in account the inter-individual cluster
24
25 211 variability, univariate associations based on regression curve analysis was performed. The
26
27 212 curve (linear, quadratic, cubic, logarithmic and power) that explained the greatest proportion of
28
29 213 the variance in tenderness scores (\pm SD) with each mean cluster variable from the 4 levels of
30
31 214 the continuum was taken as the model of best fit. The significant differences were considered
32
33 215 at a significance level of $P < 0.05$.

31 216 RESULTS

33 217 *Characteristics of the clusters for the farmgate-to-fork continuum data*

34 218 The results of the HCA are represented graphically with a dendrogram²⁴ (**Fig. 1A**), and
35
36 219 confirmed using multidimensional scaling analysis on the Euclidean distances (**Fig. 1B**).
37
38 220 Thanks to the Euclidean distances and the Ward's minimum variance method, 10 clusters were
39
40 221 retained. The individual scores of the first two axis averaged per cluster confirmed the
41
42 222 similarities and divergences among the 10 clusters (**Fig. 1C**). Therefore, the distribution of the
43
44 223 animals in the first two dimensions (**Fig. 1B**) explaining more than 75% of the variability
45
46 224 emphasized the strong inter-individual variability that exist among the animal clusters.

47
48 225 For the data at each level of the continuum, more differences than similarities were observed
49
50 226 among the clusters (**Tables 3 and 4**). Firstly, significant differences ($P < 0.0001$) were observed
51
52 227 for the 4 variables (given in bold font in **Table 3**) that were used to define the 10 animal clusters.
53
54 228 At the farmgate level, the clusters 1, 2 and 10 grouped the oldest animals with ages ranging
55
56 229 from 18.7 to 19.0 months; the clusters 3, 5, 6 and 9 grouped the youngest animals with 15.0 to
57
58 230 15.4 months and the remaining 4, 7 and 8 intermediate clusters grouped those slaughtered at
59
60 231 16.9 to 17.8 months. At the slaughterhouse level, the cluster 10 with the lowest number of

232 animals ($n = 4$) grouped the heaviest carcasses, and clusters 3, 5, 6 and 9 grouped those with
233 the lightweight carcasses (ranges from 382.0 to 386.7 kg). The clusters 4, 7 and 8 that were all
234 in the same age at slaughter interval had further similar carcass weights (from 401.6 to 410.7
235 kg) and the oldest animals of clusters 1 and 2 (~440 kg) had in average +56 kg of carcass weight
236 compared to the lightest. The conformation scores (CS) of the clusters were differentiated into
237 three categories: clusters 1, 2, 4, 6 and 7 had $CS > 11$; clusters 3 and 5 had CS between 10 and
238 11 and finally the clusters 8, 9 and 10 had $CS < 10$. Similarly, fatness scores (FS) were
239 distinguished into three categories: clusters 2, 4, 5, 8, 9 and 10 with $FS > 3.0$; clusters 1 and 3
240 were between 2.5 and 3.0 and finally clusters 6 and 7 had $FS = 2.5$.

241 At the farmgate level, 7 of 8 variables including age at slaughter, were different among the
242 clusters. Initial body weight was the only variable that was not significantly different ($P > 0.05$),
243 meaning that the animals were all conducted for the finishing period with similar body weight.
244 However, the clusters were strongly different ($P < 0.001$) for the slaughter body weight
245 following the same trend than age at slaughter. It seems that the old animals were also the
246 heaviest and the inverse is true for the youngest animals. The fattening duration that strongly
247 differ ($P < 0.001$) among the clusters can be subdivided into 5 categories. The clusters 1 and 2
248 that grouped the oldest and heaviest animals were further finished at the longest fattening
249 duration (> 270 days). Inversely, the animals of the cluster 10 were fattened at the lowest
250 duration (112 days). Most clusters, namely 4, 5, 6 and 7 were finished at the intermediate
251 fattening duration of 173 to 193 days. The animals of the clusters 3 and 9 were finished at 163
252 to 167 days and finally the cluster 8 grouped those finished at 219 days. Dry matter intake
253 (DMI) is another rearing factor that differentiated the clusters and sorted them according to
254 three thresholds: > 10 kg DM/day; between 9 and 10 kg DM/day and < 9 kg DM/day. Five
255 clusters, namely 1, 2, 3, 6 and 7 belong to the first category with mean values from 10.3 to 11.1
256 kg DM/day. Clusters 4, 5 and 8 had DMI of 9.3 to 9.5 kg DM/day. The clusters 9 and 10 had
257 the lowest DMI of 8.3 kg DM/day.

258 At the slaughterhouse level and for the other carcass traits (**Table 3**), fat and muscle carcass
259 weights as well as dressing per cent were significantly different ($P < 0.05$). For fat carcass
260 weight, the cluster 10 and 9 had respectively the highest values of 83.4 and 72.6 kg. The other
261 clusters were between 50 and 70 kg. Clusters 2, 5 and 8 had similar mean values ranging from
262 63 to 65 kg and clusters 1, 3, 4 and 6 had 53 to 59 kg. The lowest fat carcass weight of 49.2 kg
263 was found for the animals of cluster 7. Inversely, muscle carcass weight was found to be the
264 highest in the clusters that had low fat carcass weight. Dressing per cent of the carcasses varied

265 weakly among the clusters from 57.4 to 60.5 per cent. Cluster 7 grouped the animals with the
266 greatest and cluster 2 those with the lowest mean values.

267 At the muscle level, only insoluble collagen content and ICDH activity were significantly
268 different ($P < 0.05$) among the 10 clusters (**Table 4**). The lowest values of insoluble collagen
269 content (2.63 to 2.79 $\mu\text{g OH-prol mg}^{-1}$ DM) were found in clusters 9 and 10 and the highest
270 value (3.63 $\mu\text{g OH-prol mg}^{-1}$ DM) was in cluster 6. The remaining clusters had similar mean
271 values ranging from 2.93 to 3.45 $\mu\text{g OH-prol mg}^{-1}$ DM. For ICDH, the cluster 10 alone had the
272 highest enzyme activity, followed by clusters 1, 2, 6 and 9 with mean values of 1.21 to 1.39
273 $\mu\text{mol min}^{-1} \text{g}^{-1}$ and then the clusters 3, 4, 5, 7 and 8 with mean values of 1.06 to 1.19 $\mu\text{mol min}^{-1}$
274 g^{-1} .

275 At the meat level, 4 of the 5 sensory and technological/chemical meat quality traits were
276 significantly different ($P < 0.01$) among the clusters (**Table 4**). Ultimate pH with mean values
277 ranging from 5.55 to 5.60 is the only variable that did not discriminate the clusters ($P > 0.05$).
278 It is worth emphasizing that individual values that were > 5.8 or > 6.0 counted for 2.3 per cent
279 and 0.58 per cent of cattle in our dataset, respectively. For the sensory attributes, the cluster 9
280 grouped the steaks that were tender, juicy and more flavorful compared to the other clusters.
281 Finally, intramuscular fat content was not found the highest in clusters 9 and 10 that were
282 tender, juicy and flavorful but in the clusters 3 and 5 that were intermediate for these sensory
283 traits. The differences and similarities among the 10 clusters for the whole variables of the
284 continuum data are further summarized in **Fig. 2** based on an unsupervised hierarchical
285 clustering heatmap.

286 *Inter-individual cluster variability and associations between mean tenderness scores with* 287 *rearing factors*

288 The rearing factors were regressed with the mean tenderness scores to study the inter-
289 individual cluster variability and the type and direction of the associations (**Fig. 3**). The plots
290 highlighted that seven of eight rearing factors were significantly correlated to meat tenderness
291 in linear or curvilinear direction. Strong inter-individual variability exists among clusters for
292 each rearing factor. In agreement with the non-significant effect observed for initial body
293 weight, no correlation was found with tenderness (**Table 3**). However, concentrate per cent (r^2
294 = 0.81) and energy intake ($r^2 = 0.39$) were positively linked with mean tenderness scores.
295 Inversely to these positive links, age at slaughter ($r^2 = 0.24$), fattening duration ($r^2 = 0.36$),
296 slaughter body weight ($r^2 = 0.53$), forage per cent ($r^2 = 0.81$) and DMI ($r^2 = 0.36$) were all
297 negatively associated with tenderness. It seems that the old and heaviest animals that were

1
2
3 298 fattened for a long period with high content of forage and DMI, gave tough meat in comparison
4
5 299 to the youngest and lightweight animals that were fattened at durations ranging from 160 and
6
7 300 170 days with >50 per cent of concentrate and 16 Mcal of energy intake/day. Besides the
8
9 301 comparison of the extreme differences that showed strong inter-individual variability due to
10
11 302 multifactorial effects at the farmgate level, the cluster 4 highlighted the medium situation for
12
13 303 both carcass traits and tenderness. More precisely, the slaughtering of animals belonging to
14
15 304 cluster 4 at an average of 17 months after a fattening duration of approximately 190 days with
16
17 305 55 per cent of concentrate and 9 kg DM/day of DMI are likely to give tender meat with
18
19 306 interesting carcass characteristics (**Fig. 4**).

19 307 ***Inter-individual cluster variability and associations between mean tenderness scores with***
20
21 308 ***carcass characteristics***

22
23 309 The six carcass characteristics were all associated with meat tenderness and most of them in
24
25 310 a curvilinear manner (**Fig. 4**). The greatest are carcass and muscle carcass weights (clusters 1,
26
27 311 2 and 10) the toughest are the steaks ($r^2 = 0.41 - 0.56$). The inverse was true for clusters 3, 5
28
29 312 and 9, which grouped exclusively the tender steaks. However, fatness scores of the carcasses,
30
31 313 fat carcass weight and dressing per cent were best described by quadratic Bezier curves. For
32
33 314 dressing per cent, the highest (> 60 per cent) and lowest (< 58 per cent) values gave tough meat
34
35 315 and these concern cluster 7 for the former and clusters 2 and 6 for the latter. The clusters 3, 4,
36
37 316 5, 8 and 9 but not 1 and 10 that had dressing per cents between 58 and 60 gave all tender steaks.
38
39 317 The same five clusters 3, 4, 5, 8 and 9 that grouped the tender steaks were further at the top of
40
41 318 the curves for fat carcass weight (if > 58 kg and < 65 kg) and fatness score (if > 2.8 and ≤ 3.2).
42
43 319 Thus, the clusters that had fat carcass weight greater than 65 (clusters 2 and 10) or below 55 kg
44
45 320 (6 and 7) gave tough meat including the clusters 1 and 2 (**Fig. 4**). The same trend was found for
46
47 321 fatness score, another estimate of fat carcass content. Finally, conformation score was linked
48
49 322 with tenderness following the three categories discussed above. The more tender steaks were
50
51 323 those grouped in cluster 9 with the lowest conformation score and the toughest steaks are those
52
53 324 of clusters 1, 2, 6 and 7. The ordered following clusters 8, 5, 3 and 4 were positively linked with
54
55 325 tenderness and had scores varying from 4.9 to 5.4.

53 326 ***Inter-individual cluster variability and associations between mean tenderness scores with***
54
55 327 ***muscle characteristics and meat quality traits***

56
57 328 The results of the relationships among muscle characteristics (enzyme activities and
58
59 329 connective tissue properties) and meat quality traits (ultimate pH and intramuscular) showed a
60
330 weak correlation of insoluble collagen only with tenderness scores ($r^2 = 0.26$, **Fig. 5**). The

1
2
3 331 clusters that had insoluble collagen greater than 3.2 $\mu\text{g OH-prol mg}^{-1}$ DM grouped the tough
4
5 332 steaks but not the youngest animals of clusters 3 and 5.

6
7 333 ***Between-cluster principal component analysis and associations among the whole***
8
9 334 ***variables of the continuum from farmgate-to-fork***

10
11 335 The summary of the above-described relationships within each cluster and for each level of
12
13 336 the continuum are highlighted in **Fig. 6**. This between-cluster principal component analysis
14
15 337 allowed identifying the location of animal groups and variables of each level of the continuum
16
17 338 data to achieve an objective carcass or meat quality (**Fig. 6**). The similar individuals by their
18
19 339 variability (the closest are grouped together) can be easily characterized from the left to the
20
21 340 right of the graph within each level of the continuum. For example, the similar individuals of
22
23 341 cluster 9 on the top left of the graph grouped the more tender, juicy and flavorful steaks (meat
24
25 342 level). This cluster is characterized at the farmgate level by the highest energy intake,
26
27 343 concentrate per cent and initial body weight and at the same time with the lowest dry matter
28
29 344 intake, fattening duration, forage per cent, slaughter body weight, and age at slaughter. At the
30
31 345 slaughterhouse level, the carcasses had the highest fatness score but the lowest conformation
32
33 346 score and muscle carcass weight. At the muscle level, the steaks of cluster 9 had the lowest
34
35 347 insoluble collagen but with similar pHu and IMF content at the meat level compared to the
36
37 348 clusters positioned in the bottom (cluster 1 and 2). However, for the animals grouped in the
38
39 349 clusters 1 or 2 compared to individuals of cluster 9 the inverse was observed. Overall, this
40
41 350 between-cluster principal component analysis highlights the extent of the inter-individual
42
43 351 variability that exist in a selected population of cattle based on the animal characteristics and
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45 352 EUROP grid criteria fixed by the slaughterers.

46
47 353 **DISCUSSION**

48
49 354 One of the problems facing both researchers and beef industry is the handling of the
50
51 355 individual differences among animals²⁵ to offer consistent meat quality including tenderness.
52
53 356 Taken together, the results of the present study are indicative of the inter-individual cluster
54
55 357 variability rising in tenderness in link with continuum data for the closest individuals grouped
56
57 358 according to their age at slaughter and carcass properties under EUROP grid. It seems that
58
59 359 considering the continuum data would allow possible trade-off managements of tenderness to
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61 360 identify levers at different levels from the farmgate-to-meat. However, the variables
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63 361 contributing most to the inter-individual cluster variability of tenderness seemed to be more
64
65 362 related to the rearing practices, specifically feeding and their consequences on carcass
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67 363 properties than to muscle characteristics. These confirm well our previous investigations on

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3 364 young bulls using unsupervised learning and clustering methods⁵ as well as decision trees.¹¹ On
4
5 365 another hand and in agreement to the statements by Lawrence and co-workers, the data
6
7 366 highlighted that animals subjected to different planes of nutrition, even if they are without breed
8
9 367 effect in this study or selected in close thresholds such as age or weight, differ in form,
10
11 368 composition, muscle characteristics and final quality of meat²⁶.

12 369 As expected and in line with earlier studies, age at slaughter was a main source of
13
14 370 variability,²⁷ although the narrow range (CV = 10.6%) of the slaughter age of the animals. In
15
16 371 this study, meat from the youngest (<15.5 months) and lightweight bulls (< 660 kg) was more
17
18 372 tender than the oldest (> 18.5 months) and heaviest bulls (740 kg). These differences are in
19
20 373 agreement with the negative correlation observed between animal age and tenderness.^{5, 28} If we
21
22 374 refer to earlier reports, some authors attributed differences in tenderness to a potential increase
23
24 375 in connective tissue by collagen content with increasing age at slaughter as well as
25
26 376 animal/carcass maturity.^{29, 30} Tenderness in lean meat is usually associated with the collagen of
27
28 377 muscles.³¹ Of course, this was partly confirmed by our data as insoluble but not total collagen
29
30 378 was among the tenderness discriminators of the clusters. A previous study on a large dataset
31
32 379 showed that insoluble collagen was negatively correlated to tenderness.³² However, it is
33
34 380 worthwhile to note that in this study an inconsistent effect of age on insoluble collagen content
35
36 381 was found for the intermediate clusters. This is difficult to interpret, but was already observed.^{27,}
37
38 382 ³³

39 383 One of the expected effects of an increase in slaughter weight is an increase in fatness
40
41 384 scores³⁴, however, we failed to confirm this and the inverse was found. Steen and Kilpatrick³⁵,
42
43 385 described that this statement would be more pronounced in heifers and steers due to hormonal
44
45 386 reasons³⁶, than in bulls where lesser extent or inverse effect can be found. The review by Owens
46
47 387 *et al*³⁷ summarized that animals fed to grow rapidly to a given age or weight as it is the case in
48
49 388 this study for certain clusters, will generally have a lower proportion of lean tissues and a higher
50
51 389 proportion of fat than those fed to grow more slowly. An earlier review and among different
52
53 390 studies, concluded that acceptable carcass weights and degrees of maturity could be achieved
54
55 391 at young ages³⁸ and our results confirm that this would further be an advantage to obtain
56
57 392 desirable beef quality (*e.g.*, tenderness). Although no breed effect was found in this study, the
58
59 393 inconsistencies would be assigned to the use of continental breeds, which differ on their
60
394 maturity and typology compared to British breeds.^{26, 36, 39} The source of variation would be
395 partly due to genetic variability that we failed to control in this trial but it is known to affect
396 both animal and growth performance as well as their carcass traits^{16, 36}.

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2
3 397 In this study, the fattiest animals (clusters) were the youngest and lightweight that were
4
5 398 finished with high amounts of concentrate and energy intake but during short fattening periods
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7 399 and with very less DMI. Steaks from average carcass fatness displayed in this trial a higher
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9 400 tenderness scores compared to those from light or very high fatness. This was further confirmed
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11 401 by fat carcass weight in agreement to the large literature.^{5, 11} However, the inverse relationships
12
13 402 between DMI and fatness scores or fat carcass weight, and consequently on tenderness is
14
15 403 controversial as it contrasts in this study with some earlier studies⁴⁰ and agrees with others⁴¹.
16
17 404 DMI is known to be linearly related with the composition of the diet⁴². Previous results by our
18
19 405 groups showed DMI as a splitter among three tenderness classes⁵ and was further retained in
20
21 406 the prediction equations of fat tissue and muscle per cent⁴³. The association of DMI with beef
22
23 407 tenderness in addition to its link with carcass characteristics were strongly supported by our
24
25 408 findings with paramount role to understand the inter-individual cluster variability. This agrees
26
27 409 with the reduction of DMI when the proportion of concentrates in the diet was increased.⁴⁴
28
29 410 From these, fattening duration, which refers to the growing of animals to a desired end-point
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31 411 can be further used as a lever proxy to achieve/manage the desirable quality of carcass or meat.
32
33 412 Indeed, we have previously validated the fattening period duration as a proxy of fat tissue and
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35 413 muscle per cents⁴³ and as a predictor of beef tenderness or flavor classes of young bulls^{5, 11, 45}.

36
37 414 A part of the variability would be further related to the energy intake for each cluster of
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39 415 individuals. Indeed, it has been reported that reducing energy intake usually decreases carcass
40
41 416 fat content⁴⁶, which could explain the lower fat content of the bulls fed with low energy intake
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43 417 and concentrate than for the counterparts clusters. Taken all together, it seems that numerous
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45 418 factors are interrelated among them. Earlier reports evidenced that carcass fat content depends
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47 419 on slaughter weight in relation to mature body size, availability of energy and nutrients and
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49 420 physiological stage affecting energy utilization.^{35, 37, 44} In this study, the different levels have
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51 421 been considered separately and a more comprehensive approach such as logistic path analysis
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53 422 can be of great interest to better described the effects on tenderness variability in a path-based
54
55 423 diagram.

56
57 424 Overall, to achieve the desirable fatness score and thus tender beef, the young bulls must be
58
59 425 more efficient under accurate feeding and duration period. The findings emphasized that the
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426 oldest and heaviest animals fattened for a long period with forage are not likely to give tender
427 meat but lead to better carcass properties *i.e.*, the heaviest with high conformation scores and
428 muscle content in the carcasses. It may be concluded that fattening young bulls to obtain a good

body condition or great carcass characteristics will not improve the qualitative merit of the final product, *i.e.*, meat tenderness potential.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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For Peer Review

Figure captions

Figure 1. Distribution of the individuals in the ten identified clusters. **A)** Dendrogram of hierarchical cluster analysis highlighting the number of the individuals in each cluster. **B)** A between-cluster principal component analysis highlighting the distribution of the individuals in the ten identified clusters. The individuals of each cluster are comprised by convex polygons as validated by the multidimensional scaling based on the 4 selected variables by industrials (age at slaughter, carcass weight, conformation and fatness scores of the carcasses) and were color-coded following those of the dendrogram. **C)** Mean scores per animal cluster on the 1st (top) and 2nd (bottom) eligible axis (eigenvalues of 1.76 and 1.25, respectively) of the projection of the 171 individuals. ^{a-g} Means \pm standard errors with no common letters are significantly different ($P < 0.05$).

Figure 2. Unsupervised hierarchical clustering heatmap highlighting the differences and similarities among the 10 clusters for the whole variables from the continuum data as separated in Tables 3 and 4. The variables concerning animal and carcass characteristics that were used to identify the clusters are shown by a black star. A color key (gradient ruler) on the upper right indicates the similarity among the clusters for each variable from the lowest LSmean value (yellow) to the highest LSmean value (red). The variables that were not significantly different among the clusters are left with a blank row.

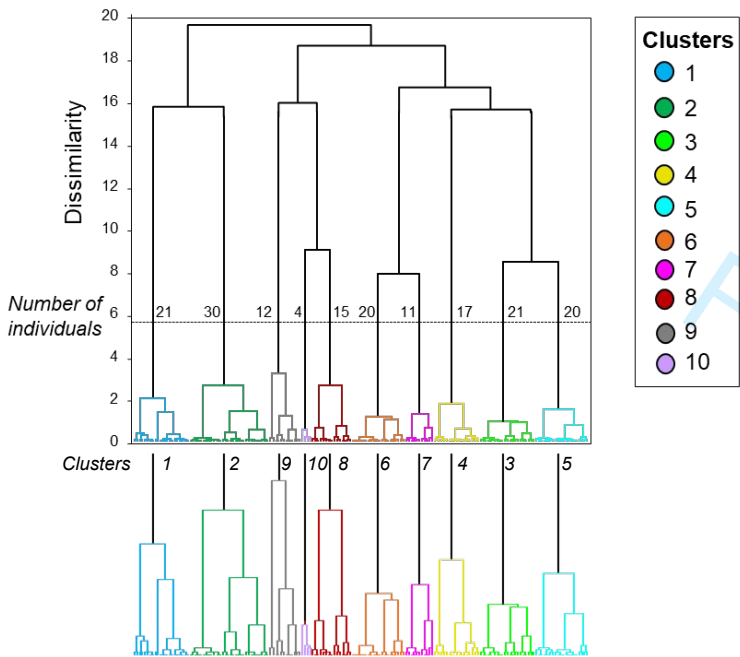
Figure 3. Correlation charts showing the individual cluster variability and associations between mean tenderness scores and rearing factors per cluster. Each point represents the mean value of each animal cluster and the error bars the individual's variability. Significant correlations by their R^2 ($P < 0.05$) are shown in bold characters.

Figure 4. Correlation charts showing the individual cluster variability and associations between mean tenderness scores and carcass characteristics per cluster. Each point represents the mean value of each animal cluster and the error bars the individual's variability. Significant correlations by their R^2 ($P < 0.05$) are shown in bold characters.

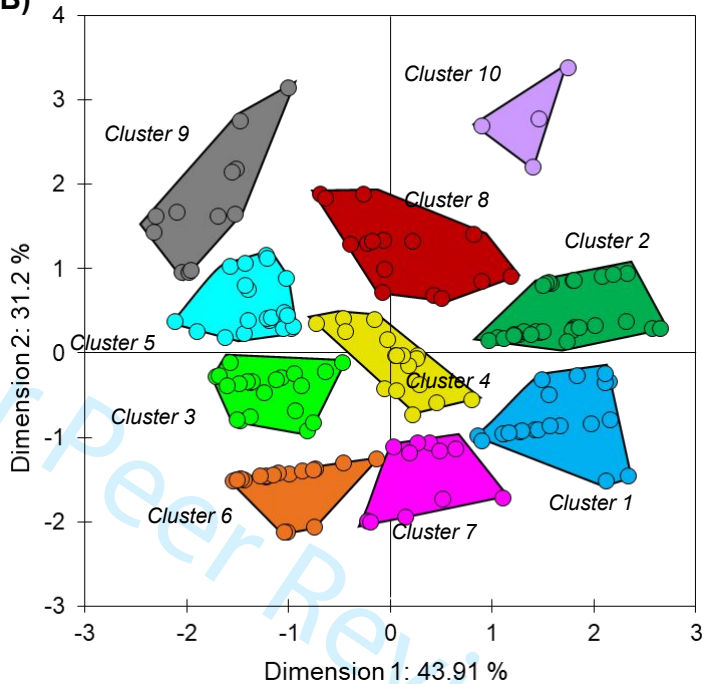
Figure 5. Correlation charts showing the individual cluster variability and associations between mean tenderness scores and muscle characteristics and meat quality traits per cluster. Each point represents the mean value of each animal cluster and the error bars the individual's variability. Significant correlations by their R^2 ($P < 0.05$) are shown in bold characters.

Figure 6. Location of animal clusters and scatterplots of individual distribution for the variables of each level of the farmgate-to-fork continuum data on the main first principal component considered (highest eigenvalue). For example, the similar individuals of cluster 9 ($n = 12$) on the top produced the more tender, juicy and flavorful steaks and characterized at the *i) farmgate level* by the highest energy intake, concentrate % and initial body weight but with the lowest dry matter intake, fattening duration, forage %, slaughter body weight, and age at slaughter; at the *ii) carcass level* by the highest fatness score and the lowest conformation score and muscle carcass weight; at the *iii) muscle level* by slightly high ICDH activity and the lowest insoluble collagen; and finally at the *iv) meat level* by similar pHu and IMF content compared to the clusters in the bottom (cluster 1). However, compared to individuals of cluster 9 the inverse was observed for the animals grouped in the clusters 1 ($n = 21$) or 2 ($n = 30$). The variables that were used to identify the animal clusters were highlighted at both farmgate and slaughterhouse levels in bold font.

A)



B)



C)

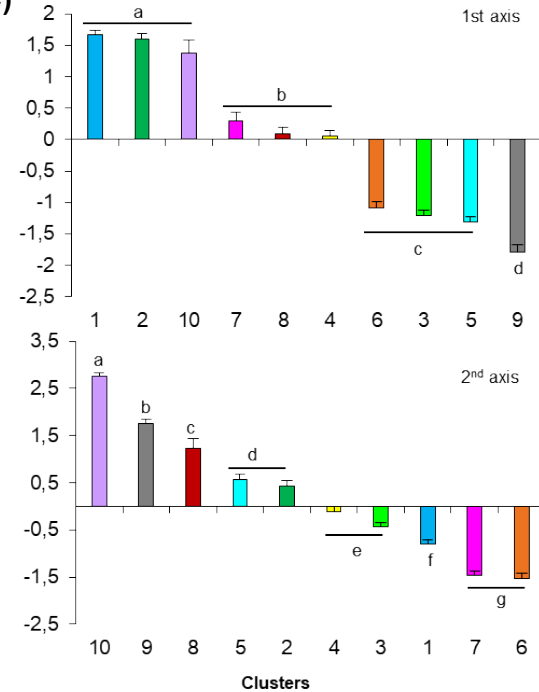


Figure 1.

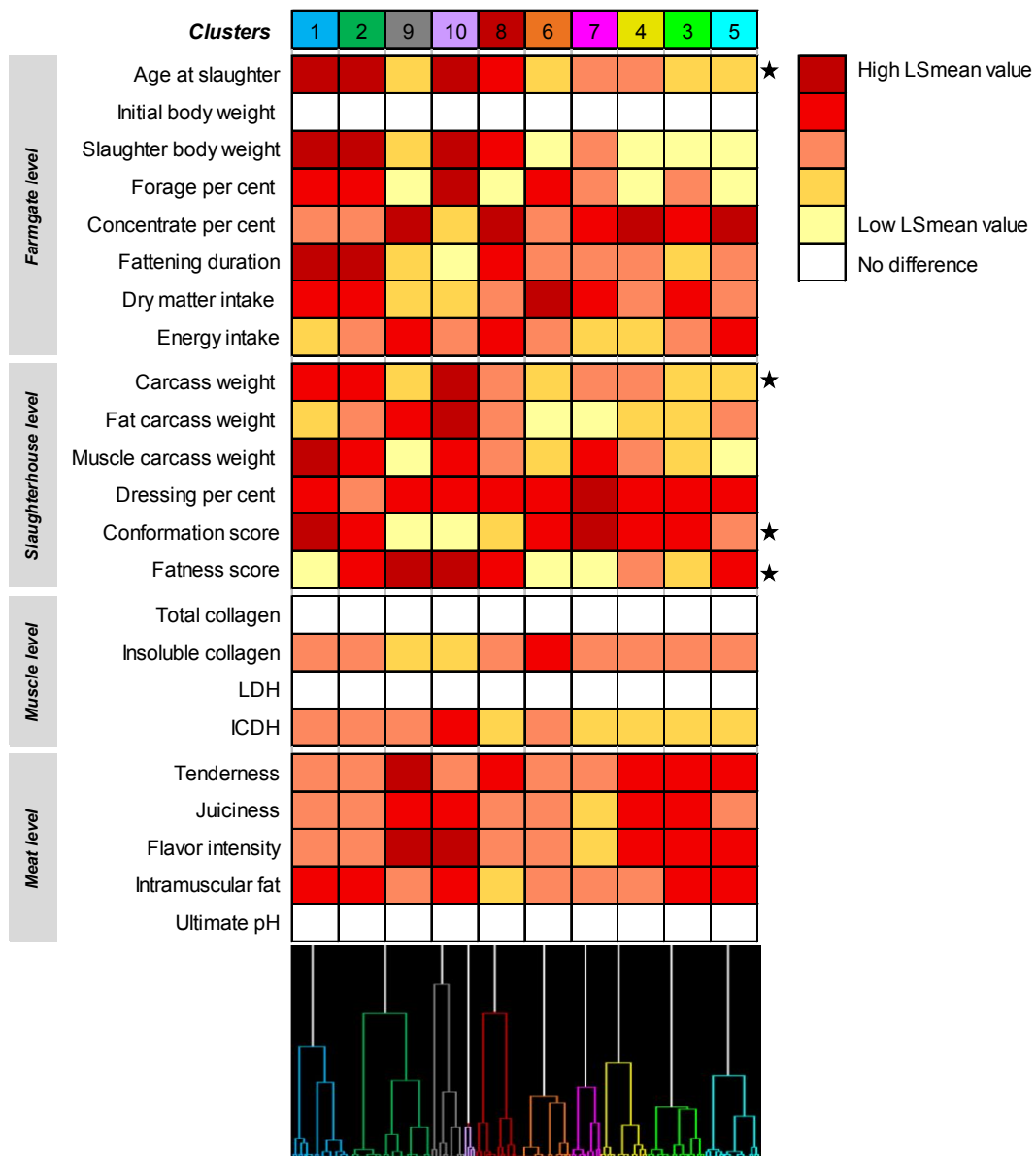


Figure 2.

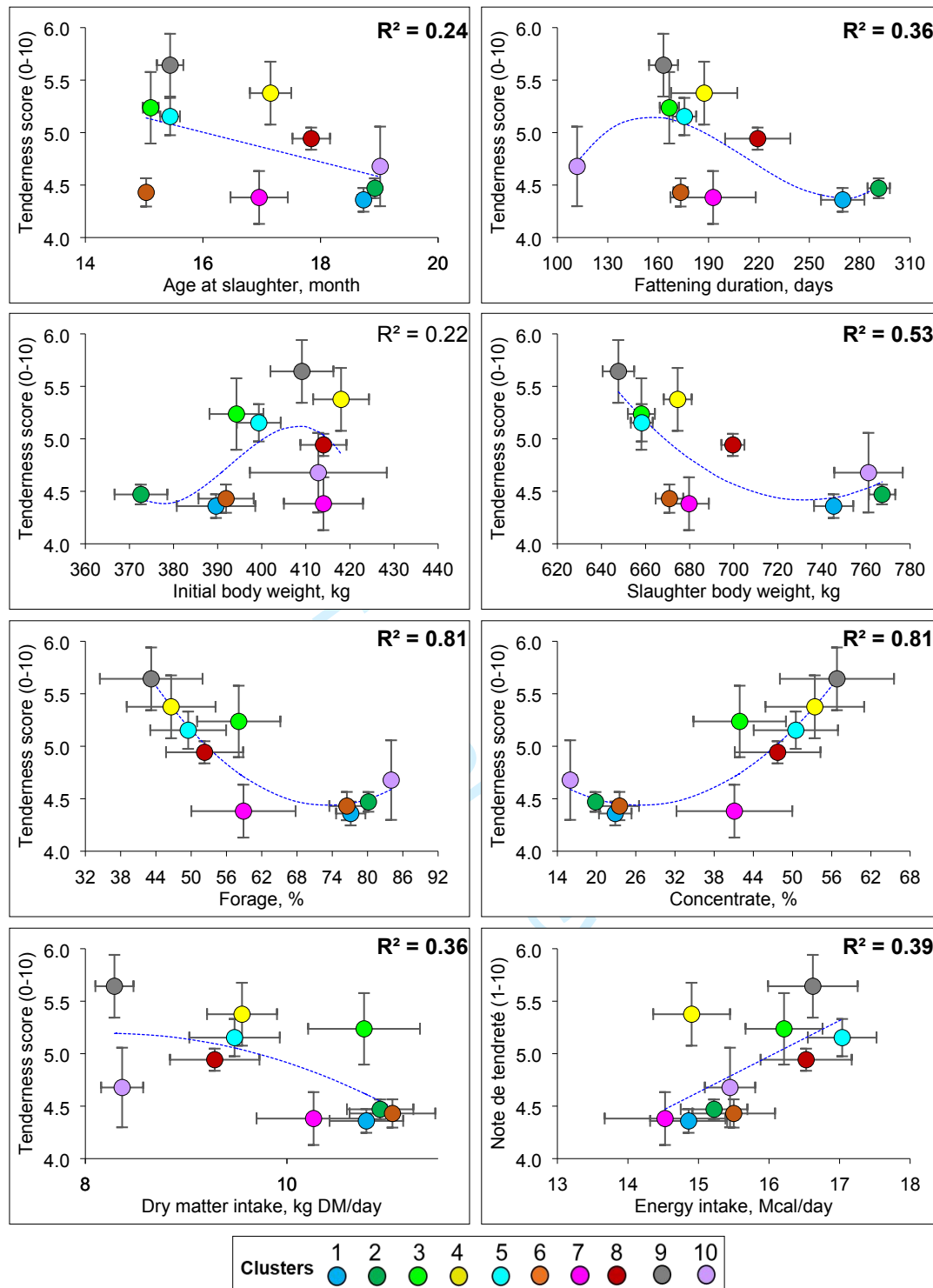


Figure 3.

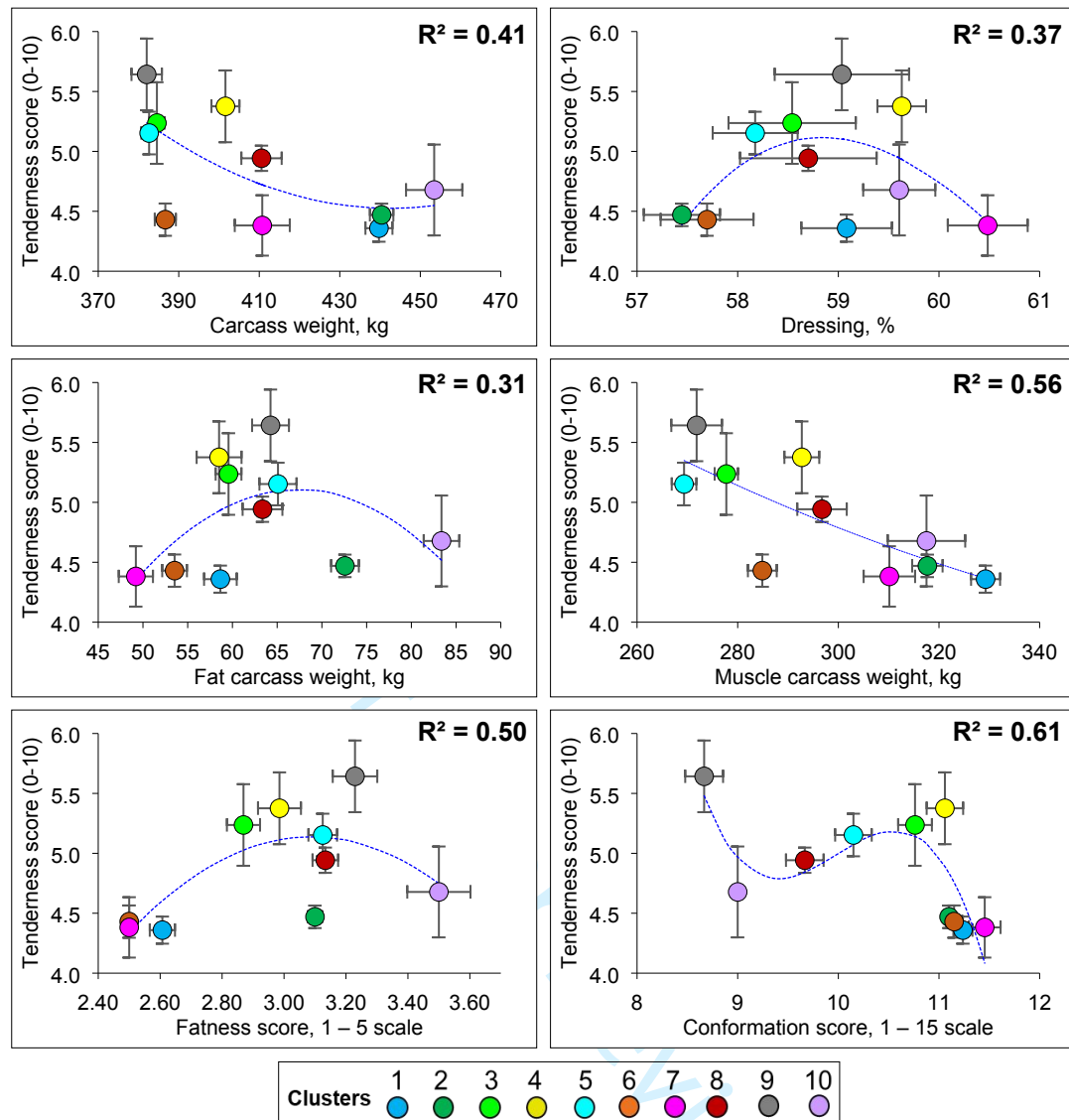


Figure 4.

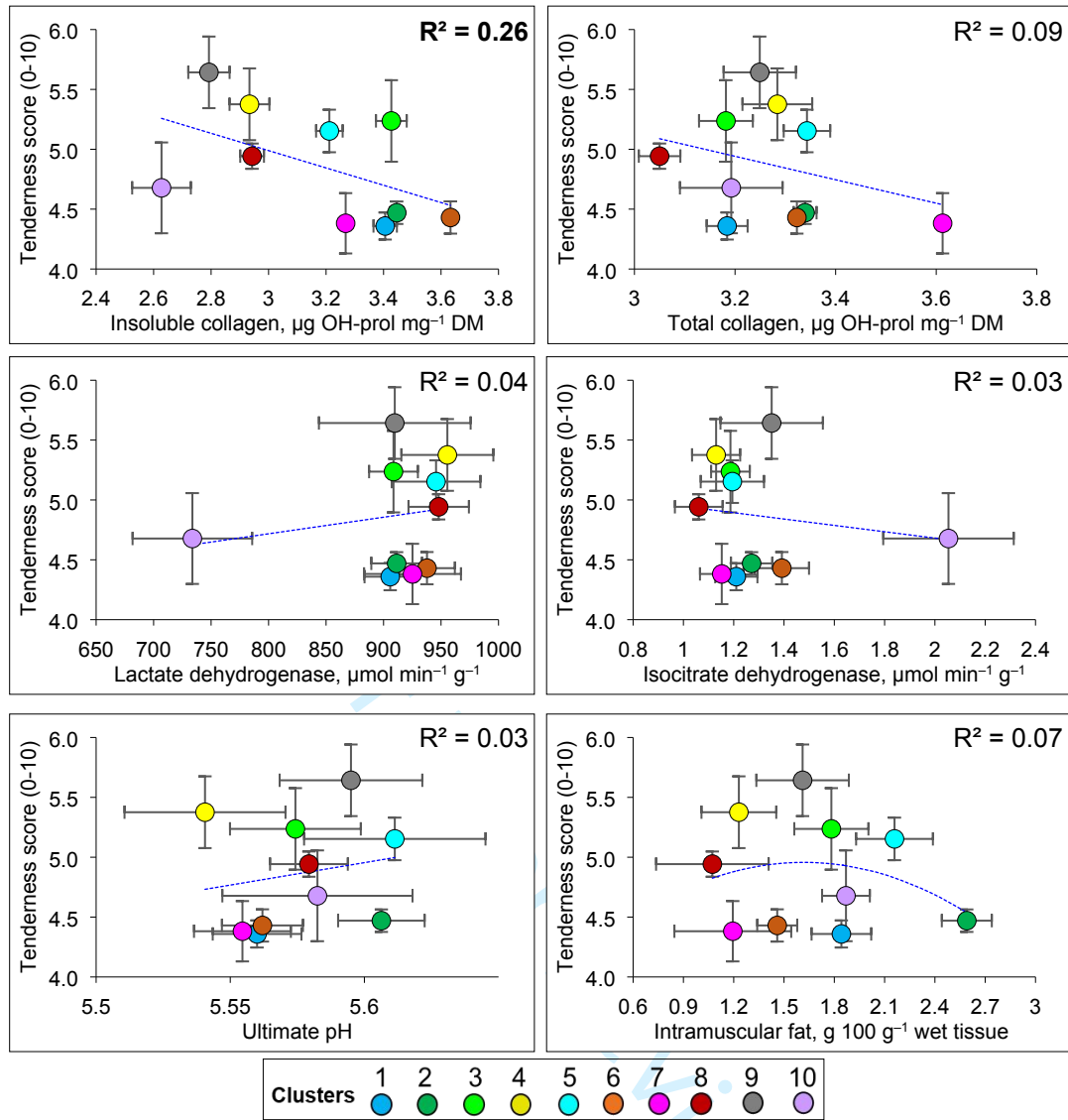


Figure 5.

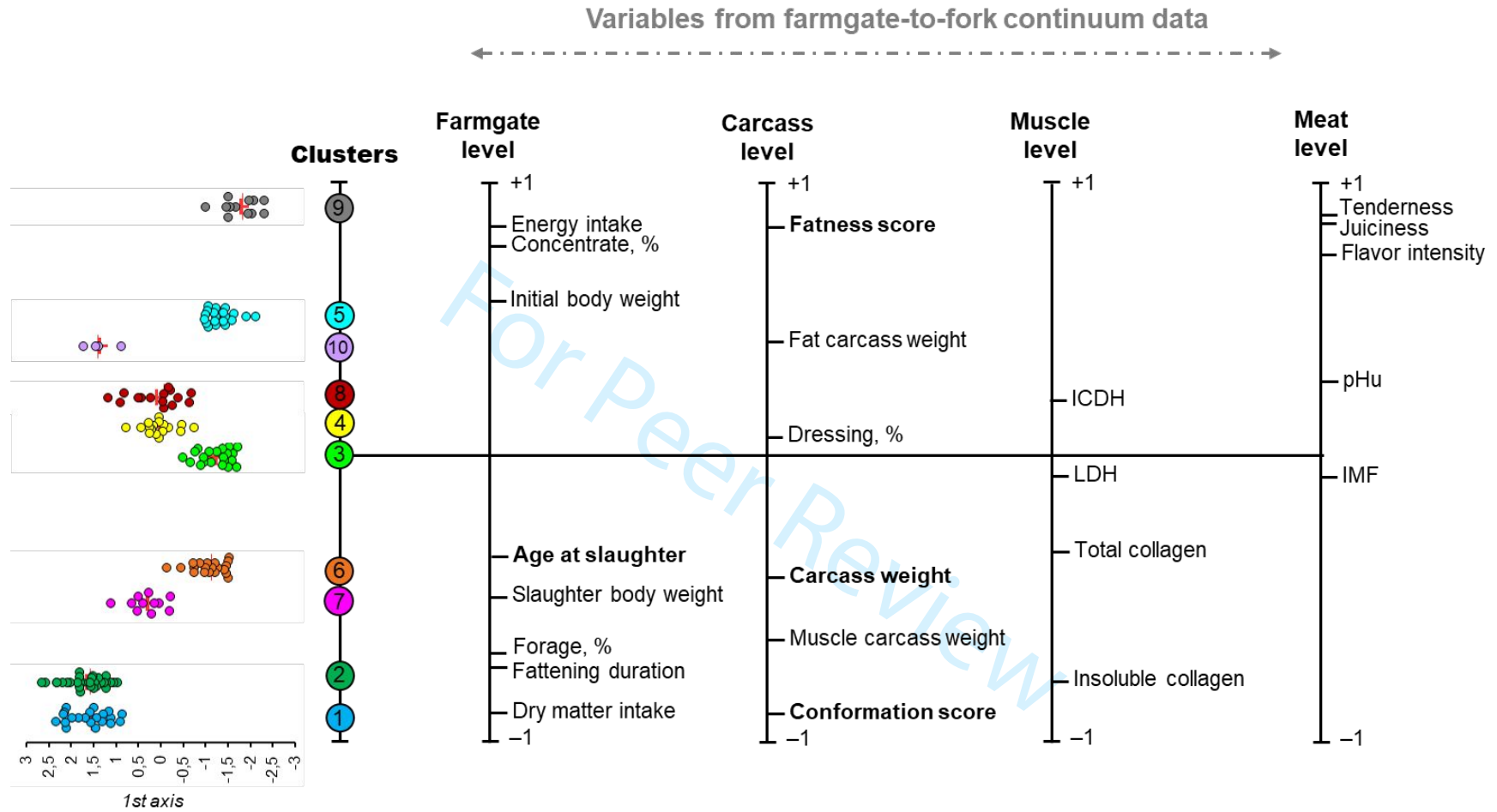


Figure 6.

Table 1. Descriptive statistics of the variables corresponding to data from farmgate level (animal characteristics and rearing factors) and slaughterhouse level (carcass characteristics and composition) measured for the 171 young bulls.

	Mean	SD	Min	Max
<i>Farmgate level: animal characteristics and rearing factors (q = 8)</i>				
Age at slaughter, month	16.93	1.79	14.03	19.58
Initial body weight, kg	397.05	55.40	276.60	567.30
Slaughter body weight, kg	697.83	52.56	608.00	841.40
Forage per cent	63.41	26.56	6.51	84.90
Concentrate per cent	36.59	26.56	15.07	93.49
Fattening duration, days	209.86	70.89	105.00	328.00
Dry matter intake, kg DM/day	10.17	1.99	6.65	14.59
Energy intake, Mcal/day	15.69	2.52	9.63	20.57
<i>Slaughterhouse level: carcass characteristics and composition (q = 6)</i>				
Carcass weight, kg	408.25	28.40	370.10	469.30
Fat carcass weight, kg ¹	62.13	10.78	36.27	92.78
Muscle carcass weight, kg ¹	296.69	25.03	246.17	359.25
Dressing per cent	58.58	2.57	52.08	67.18
Conformation score, 1 – 15 scale ²	10.64	0.98	7.00	12.00
Fatness score, 1 – 5 scale ³	2.92	0.33	2.50	3.75

¹ Muscle and fat carcass weights were estimated from the 6th rib composition after dissection¹. 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 5 = fattest.

Abbreviations: SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 2. Descriptive statistics of the variables corresponding to data from muscle level (muscle characteristics) and meat level (meat quality traits) measured for the 171 young bulls in *Longissimus thoracis* muscle.

	Mean	SD	Min	Max
<i>Muscle level: muscle characteristics by enzyme activities and connective tissue properties (q = 4)</i>				
Total collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	3.28	0.72	2.03	5.48
Insoluble collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$	3.26	0.77	1.83	5.74
Lactate dehydrogenase (LDH), $\mu\text{mol min}^{-1}\text{ g}^{-1}$	921.68	138.55	555.90	1401.50
Isocitrate dehydrogenase (ICDH), $\mu\text{mol min}^{-1}\text{ g}^{-1}$	1.24	0.47	0.40	2.76
<i>Meat level: sensory and technological/chemical meat quality traits (q = 5)</i>				
Tenderness, 0 – 10 scale	4.84	1.00	1.77	8.63
Juiciness, 0 – 10 scale	4.77	0.85	2.63	8.00
Flavor intensity, 0 – 10 scale	4.75	0.80	3.38	7.00
Intramuscular fat, $\text{g } 100\text{ g}^{-1}\text{ wet tissue}$	17.73	10.31	0.91	44.96
Ultimate pH	5.58	0.10	5.32	6.01

Abbreviations: SD: Standard deviation; Min: Minimum; Max: Maximum.

Table 3. Variance analysis highlighting differences among the ten clusters for data from farmgate level (animal characteristics and rearing factors) and slaughterhouse level (carcass characteristics and composition) measured for the 171 young bulls.

Variables ¹	n =	Clusters										SEM	P-value ²
		1 (21)	2 (30)	3 (21)	4 (17)	5 (20)	6 (20)	7 (11)	8 (15)	9 (12)	10 (4)		
<i>Farmgate level: animal characteristics and rearing factors</i>													
Age at slaughter, month³		18.7 ^a	18.9 ^a	15.1 ^d	17.2 ^c	15.4 ^d	15.0 ^d	16.9 ^c	17.8 ^b	15.4 ^d	19.0 ^a	0.20	***
Initial body weight, kg		389.6	372.6	394.3	418.0	399.3	391.9	414.0	414.0	409.1	412.8	14.47	ns
Slaughter body weight, kg		745.3 ^a	767.3 ^a	658.1 ^{cd}	674.6 ^{cd}	658.3 ^{cd}	670.8 ^{cd}	679.7 ^c	699.6 ^b	647.7 ^d	761.1 ^a	7.54	***
Forage per cent		77.1 ^{ab}	80.2 ^{ab}	58.1 ^{bc}	46.6 ^c	49.5 ^c	76.5 ^{ab}	58.9 ^{bc}	52.3 ^c	43.2 ^c	84.0 ^a	5.17	***
Concentrate per cent		22.9 ^{bc}	19.8 ^{bc}	41.9 ^{ab}	53.4 ^a	50.5 ^a	23.5 ^{bc}	41.1 ^{ab}	47.7 ^a	56.8 ^a	16.0 ^c	5.17	***
Fattening duration, days		269.9 ^a	291.3 ^a	166.6 ^c	187.4 ^{bc}	175.6 ^{bc}	173.4 ^{bc}	192.7 ^{bc}	219.3 ^b	163.2 ^c	111.7 ^d	11.30	***
Dry matter intake, kg DM/day		10.8 ^a	10.9 ^a	10.8 ^a	9.5 ^{ab}	9.5 ^{ab}	11.1 ^a	10.3 ^a	9.3 ^{ab}	8.3 ^b	8.3 ^b	0.39	**
Energy intake, Mcal/day		14.9 ^c	15.2 ^b	16.2 ^{ab}	14.9 ^c	17.0 ^a	15.5 ^b	14.5 ^c	16.5 ^a	16.6 ^a	15.4 ^b	0.57	*
<i>Slaughterhouse level: carcass characteristics and composition</i>													
Carcass weight, kg³		439.7 ^b	440.4 ^b	384.6 ^d	401.6 ^c	382.6 ^d	386.7 ^d	410.7 ^c	410.6 ^c	382.0 ^d	453.4 ^a	3.88	***
Fat carcass weight, kg		58.7 ^{cd}	64.3 ^c	59.6 ^{cd}	58.5 ^{cd}	65.1 ^c	53.5 ^{de}	49.2 ^e	63.4 ^c	72.6 ^b	83.4 ^a	1.90	***
Muscle carcass weight, kg		329.3 ^a	317.7 ^{ab}	277.7 ^{de}	292.7 ^c	269.3 ^e	284.9 ^{cd}	310.1 ^b	296.7 ^c	271.8 ^e	317.5 ^{ab}	3.98	***
Dressing per cent		59.1 ^{ab}	57.4 ^b	58.5 ^{ab}	59.6 ^{ab}	58.2 ^{ab}	57.7 ^{ab}	60.5 ^a	58.7 ^{ab}	59.0 ^{ab}	59.6 ^{ab}	0.47	*
Conformation score, 1 – 15 scale³		11.2 ^{ab}	11.1 ^{ab}	10.7 ^b	11.1 ^{ab}	10.1 ^c	11.1 ^{ab}	11.4 ^a	9.7 ^d	8.7 ^e	9.0 ^e	0.13	***
Fatness score, 1 – 5 scale³		2.6 ^e	3.1 ^{bc}	2.87 ^d	3.0 ^c	3.1 ^{bc}	2.5 ^e	2.5 ^e	3.1 ^{bc}	3.2 ^b	3.5 ^a	0.16	***

¹ Least-square means in the same row with different superscript letters are significantly different ($P < 0.05$).

² Significances: ns: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

³ Variables in bold characters highlight those used to construct the clusters based on the 4 criteria fixed by the beef sector.

Table 4. Variance analysis highlighting differences among the ten clusters for data from muscle level (muscle characteristics) and meat level (meat quality traits) measured for the 171 young bulls in *Longissimus thoracis* muscle.

Variables	n =	Clusters										SEM	P-value
		1 (21)	2 (30)	3 (21)	4 (17)	5 (20)	6 (20)	7 (11)	8 (15)	9 (12)	10 (4)		
<i>Muscle level: muscle characteristics by enzyme activities and connective tissue properties</i>													
Total collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$		3.18	3.34	3.18	3.28	3.34	3.32	3.61	3.05	3.25	3.19	0.19	ns
Insoluble collagen, $\mu\text{g OH-prol mg}^{-1}\text{ DM}$		3.41 ^{ab}	3.45 ^{ab}	3.43 ^{ab}	2.93 ^{ab}	3.21 ^{ab}	3.63 ^a	3.27 ^{ab}	2.94 ^{ab}	2.79 ^b	2.63 ^b	0.20	**
Lactate dehydrogenase (LDH), $\mu\text{mol min}^{-1}\text{ g}^{-1}$		905.8	911.4	908.6	955.5	945.7	937.8	925.2	947.9	909.8	733.7	35.48	ns
Isocitrate dehydrogenase (ICDH), $\mu\text{mol min}^{-1}\text{ g}^{-1}$		1.21 ^b	1.27 ^b	1.19 ^c	1.13 ^c	1.19 ^c	1.39 ^b	1.15 ^c	1.06 ^c	1.35 ^b	2.05 ^a	0.12	*
<i>Meat level: sensory and technological/chemical meat quality traits</i>													
Tenderness, 0 – 10 scale		4.4 ^b	4.5 ^b	5.2 ^{ab}	5.4 ^{ab}	5.2 ^{ab}	4.4 ^b	4.4 ^b	4.9 ^{ab}	5.6 ^a	4.7 ^b	0.22	***
Juiciness, 0 – 10 scale		4.5 ^{ab}	4.5 ^{ab}	5.1 ^a	5.1 ^a	4.8 ^{ab}	4.6 ^{ab}	4.2 ^b	4.8 ^{ab}	5.3 ^a	5.4 ^a	0.18	**
Flavor intensity, 0 – 10 scale		4.5 ^{bcd}	4.9 ^{abc}	4.9 ^{abc}	4.8 ^{abc}	5.2 ^{ab}	4.3 ^{cd}	4.0 ^d	4.5 ^{bcd}	5.4 ^a	5.3 ^a	0.17	***
Intramuscular fat, g 100 g ⁻¹ wet tissue		1.84 ^{abc}	2.59 ^a	1.78 ^{abc}	1.23 ^{bc}	2.16 ^{ab}	1.46 ^{bc}	1.19 ^{bc}	1.07 ^c	1.61 ^{bc}	1.87 ^{abc}	0.08	***
Ultimate pH		5.56	5.61	5.57	5.54	5.61	5.56	5.55	5.58	5.60	5.58	0.02	ns

¹ Least-square means in the same row with different superscript letters are significantly different ($P < 0.05$).

² Significances: ns: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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4 **continuum data from the farmgate-to-fork for ultimate beef tenderness**
5 **management**
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30 **Appendices**
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35 **Table S1.** Breed effect on the 4 animal and carcass characteristics criteria used for the selection
36 of the 171 young bulls from the large INRA database of 480 animals; The selection was based
37 on the attempts (ranges) fixed by the slaughterhouses surveyed in the Auvergne-Rhône-Alpes
38 region, France.
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Table S1. Breed effect on the 4 animal and carcass characteristics criteria used for the selection of the 171 young bulls from the large INRA database of 480 animals; The selection was based on the attempts (ranges) fixed by the slaughterhouses surveyed in the Auvergne-Rhône-Alpes region, France.

Variables	Breeds			SEM	<i>P</i> -value
	Charolais (n = 124)	Limousin (n = 24)	Salers (n = 23)		
Age at slaughter, months	17.08	16.43	16.78	0.30	0.27
Carcass weight, kg	409.1	410.3	401.6	4.76	0.48
Conformation score, 1 – 15 scale	10.88	10.71	9.70	0.17	0.09
Fatness score, 1 – 5 scale	2.87	2.93	3.03	0.08	0.13

For Peer Review