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

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Abstract:

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

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

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

Key words: Beef tenderness; Young bulls; Carcass and muscle characteristics; Rearing factors; Multivariate analyses.

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Animals handling

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

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

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

Animal characteristics and rearing factors

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

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

Carcass characteristics and composition

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

Six parameters characterized the carcasses and their composition. First they were graded under the EU beef carcass classification scheme using experts familiar with the EUROP grid (Commission Regulation (EC) 1249/2008). Five conformation classes were defined, represented by the letters E, U, R, O, and P. The scoring consists of a visual assessment of carcass muscling where carcasses graded as 'E' have the most muscularity, and this decreases through to 'P' which have the least muscularity. European Union regulations allow for 3 subdivisions of each conformation class, high: "+", medium: "=" and low: "-". Hence, an incremental scale ranging from 1 to 15 was used, where 1 corresponds to P- (very low muscle development) and 15 to E+ (very high muscle development)¹⁸. At the same time, the fatness score of the carcasses, which describes the amount of fat on the outside of the carcass was numerically scored from 1 = leanest to 5 = fattest. The carcass weight was measured within 2h of slaughter and was expressed as cold carcass weight, which is 0.98 times the hot carcass weight. The carcass weight was used to compute the dressing per cent and carcass composition by muscle and fat carcass weights (**Table 1**). This was estimated from the composition of the 6th rib determined after physical dissection following standard commercial practice involving a close trimming and deboning¹⁸.

Muscle characteristics by enzyme activities and connective tissue properties

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

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

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

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

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

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

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

Each sample was measured in triplicate and muscle total lipids were reported as g of fatty acid per 100 g wet tissue.

The sensory evaluation of the ribeye steaks was performed following the protocol described by Gagaoua *et al.*^{6,22}. Briefly, the frozen steaks were thawed without stacking or overlapping, at 5°C in vacuum packs for 48 h before cooking and sensory assessment at 55°C. One hour before sensory evaluation, the steaks were cut into approximately 1.50cm thick and grilled on a double grooved plate griddle (SOFRACA, Morangis, France) heated to 300°C for 30min before cooking. Steaks were heated for 2min until the end-point temperature of 55 °C in the geometric center of the steak was reached (measured using a temperature probe (Type K, HANNA HI 98704, Newark)). After grilling, each steak was cut into 20 mm cubes that were immediately served to 12 panelists²². The panelists rated the steaks on a 10 cm unstructured line scale or the following sensory attributes: global tenderness (0, extremely tough; 10, extremely tender), juiciness (0, extremely dry; 10, extremely juicy), and beef flavor intensity (0, extremely weak; 10, extremely strong) as in Gagaoua *et al.*^{5,22}. The sensory analyses were performed in a room equipped with individual booths under artificial red light to reduce the influence of the appearance of the samples. Each tasting booth was equipped with computer terminals linked to a fileserver running a sensory software program (Fizz v 2.20 h, Biosystemes, Couternon, France) that facilitated the direct entry of assessor ratings.

Statistical analyses

A total of 23 variables including tenderness were used in this integrative study by considering the 4 levels of the continuum from farmagte-to-fork (**Tables 1** and **2**). The SAS statistical software (SAS 9.3, SAS Institute INC, Cary, NC, USA) and XLSTAT 2018.2 (AddinSoft, Paris, France) were used for data analyses. Normal distribution and homogeneity of the dataset was first tested by the Shapiro-Wilk test ($P>0.05$). Then, a general description analysis was realized by computing means, standard deviations, minimum and maximum ranges (**Tables 1** and **2**).

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

identify 10 animal clusters with less than 5% variation among them for the 4 used factors. Results of HCA are represented graphically by a dendrogram that provides a simple way of visualizing the hierarchical structure of the clustering and the level at which each cluster is formed, as well as cluster membership. The 10 animal clusters were further confirmed by multidimensional scaling (MDS) dissimilarity, an unsupervised procedure, based on the Euclidean distances matrix of the dissimilarities that were generated thanks to Young-Householder theorem²³. The 10 clusters were compared using PROC GLM procedure of SAS for the whole variables of the continuum after the projection on a new PCA to perform a between-animal cluster analysis. Therefore, we used both simple linear regression and random regression methods to model systematic changes in tenderness within clusters by considering homogeneity of variance across individuals. To take in account the inter-individual cluster variability, univariate associations based on regression curve analysis was performed. The curve (linear, quadratic, cubic, logarithmic and power) that explained the greatest proportion of the variance in tenderness scores (\pm SD) with each mean cluster variable from the 4 levels of the continuum was taken as the model of best fit. The significant differences were considered at a significance level of $P < 0.05$.

RESULTS

Characteristics of the clusters for the farmgate-to-fork continuum data

The results of the HCA are represented graphically with a dendrogram²⁴ (**Fig. 1A**), and confirmed using multidimensional scaling analysis on the Euclidean distances (**Fig. 1B**). Thanks to the Euclidean distances and the Ward's minimum variance method, 10 clusters were retained. The individual scores of the first two axis averaged per cluster confirmed the similarities and divergences among the 10 clusters (**Fig. 1C**). Therefore, the distribution of the animals in the first two dimensions (**Fig. 1B**) explaining more than 75% of the variability emphasized the strong inter-individual variability that exist among the animal clusters.

For the data at each level of the continuum, more differences than similarities were observed among the clusters (**Tables 3 and 4**). Firstly, significant differences ($P < 0.0001$) were observed for the 4 variables (given in bold font in **Table 3**) that were used to define the 10 animal clusters. At the farmgate level, the clusters 1, 2 and 10 grouped the oldest animals with ages ranging from 18.7 to 19.0 months; the clusters 3, 5, 6 and 9 grouped the youngest animals with 15.0 to 15.4 months and the remaining 4, 7 and 8 intermediate clusters grouped those slaughtered at 16.9 to 17.8 months. At the slaughterhouse level, the cluster 10 with the lowest number of

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

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

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

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

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

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

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

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

fattened for a long period with high content of forage and DMI, gave tough meat in comparison to the youngest and lightweight animals that were fattened at durations ranging from 160 and 170 days with >50 per cent of concentrate and 16 Mcal of energy intake/day. Besides the comparison of the extreme differences that showed strong inter-individual variability due to multifactorial effects at the farmgate level, the cluster 4 highlighted the medium situation for both carcass traits and tenderness. More precisely, the slaughtering of animals belonging to cluster 4 at an average of 17 months after a fattening duration of approximately 190 days with 55 per cent of concentrate and 9 kg DM/day of DMI are likely to give tender meat with interesting carcass characteristics (**Fig. 4**).

Inter-individual cluster variability and associations between mean tenderness scores with carcass characteristics

The six carcass characteristics were all associated with meat tenderness and most of them in a curvilinear manner (**Fig. 4**). The greatest are carcass and muscle carcass weights (clusters 1, 2 and 10) the toughest are the steaks ($r^2 = 0.41 - 0.56$). The inverse was true for clusters 3, 5 and 9, which grouped exclusively the tender steaks. However, fatness scores of the carcasses, fat carcass weight and dressing per cent were best described by quadratic Bezier curves. For dressing per cent, the highest (> 60 per cent) and lowest (< 58 per cent) values gave tough meat and these concern cluster 7 for the former and clusters 2 and 6 for the latter. The clusters 3, 4, 5, 8 and 9 but not 1 and 10 that had dressing per cents between 58 and 60 gave all tender steaks. The same five clusters 3, 4, 5, 8 and 9 that grouped the tender steaks were further at the top of the curves for fat carcass weight (if > 58 kg and < 65 kg) and fatness score (if > 2.8 and ≤ 3.2). Thus, the clusters that had fat carcass weight greater than 65 (clusters 2 and 10) or below 55 kg (6 and 7) gave tough meat including the clusters 1 and 2 (**Fig. 4**). The same trend was found for fatness score, another estimate of fat carcass content. Finally, conformation score was linked with tenderness following the three categories discussed above. The more tender steaks were those grouped in cluster 9 with the lowest conformation score and the toughest steaks are those of clusters 1, 2, 6 and 7. The ordered following clusters 8, 5, 3 and 4 were positively linked with tenderness and had scores varying from 4.9 to 5.4.

Inter-individual cluster variability and associations between mean tenderness scores with muscle characteristics and meat quality traits

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

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

Between-cluster principal component analysis and associations among the whole variables of the continuum from farmgate-to-fork

The summary of the above-described relationships within each cluster and for each level of the continuum are highlighted in **Fig. 6**. This between-cluster principal component analysis allowed identifying the location of animal groups and variables of each level of the continuum data to achieve an objective carcass or meat quality (**Fig. 6**). The similar individuals by their variability (the closest are grouped together) can be easily characterized from the left to the right of the graph within each level of the continuum. For example, the similar individuals of cluster 9 on the top left of the graph grouped the more tender, juicy and flavorful steaks (meat level). This cluster is characterized at the farmgate level by the highest energy intake, concentrate per cent and initial body weight and at the same time with the lowest dry matter intake, fattening duration, forage per cent, slaughter body weight, and age at slaughter. At the slaughterhouse level, the carcasses had the highest fatness score but the lowest conformation score and muscle carcass weight. At the muscle level, the steaks of cluster 9 had the lowest insoluble collagen but with similar pHu and IMF content at the meat level compared to the clusters positioned in the bottom (cluster 1 and 2). However, for the animals grouped in the clusters 1 or 2 compared to individuals of cluster 9 the inverse was observed. Overall, this between-cluster principal component analysis highlights the extent of the inter-individual variability that exist in a selected population of cattle based on the animal characteristics and EUROP grid criteria fixed by the slaughterers.

DISCUSSION

One of the problems facing both researchers and beef industry is the handling of the individual differences among animals²⁵ to offer consistent meat quality including tenderness. Taken together, the results of the present study are indicative of the inter-individual cluster variability rising in tenderness in link with continuum data for the closest individuals grouped according to their age at slaughter and carcass properties under EUROP grid. It seems that considering the continuum data would allow possible trade-off managements of tenderness to identify levers at different levels from the farmgate-to-meat. However, the variables contributing most to the inter-individual cluster variability of tenderness seemed to be more related to the rearing practices, specifically feeding and their consequences on carcass properties than to muscle characteristics. These confirm well our previous investigations on

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

As expected and in line with earlier studies, age at slaughter was a main source of variability,²⁷ although the narrow range (CV = 10.6%) of the slaughter age of the animals. In this study, meat from the youngest (<15.5 months) and lightweight bulls (< 660 kg) was more tender than the oldest (> 18.5 months) and heaviest bulls (740 kg). These differences are in agreement with the negative correlation observed between animal age and tenderness.^{5, 28} If we refer to earlier reports, some authors attributed differences in tenderness to a potential increase in connective tissue by collagen content with increasing age at slaughter as well as animal/carcass maturity.^{29, 30} Tenderness in lean meat is usually associated with the collagen of muscles.³¹ Of course, this was partly confirmed by our data as insoluble but not total collagen was among the tenderness discriminators of the clusters. A previous study on a large dataset showed that insoluble collagen was negatively correlated to tenderness.³² However, it is worthwhile to note that in this study an inconsistent effect of age on insoluble collagen content was found for the intermediate clusters. This is difficult to interpret, but was already observed.^{27, 33}

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

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In this study, the fattiest animals (clusters) were the youngest and lightweight that were finished with high amounts of concentrate and energy intake but during short fattening periods and with very less DMI. Steaks from average carcass fatness displayed in this trial a higher tenderness scores compared to those from light or very high fatness. This was further confirmed by fat carcass weight in agreement to the large literature.^{5, 11} However, the inverse relationships between DMI and fatness scores or fat carcass weight, and consequently on tenderness is controversial as it contrasts in this study with some earlier studies⁴⁰ and agrees with others⁴¹. DMI is known to be linearly related with the composition of the diet⁴². Previous results by our groups showed DMI as a splitter among three tenderness classes⁵ and was further retained in the prediction equations of fat tissue and muscle per cent⁴³. The association of DMI with beef tenderness in addition to its link with carcass characteristics were strongly supported by our findings with paramount role to understand the inter-individual cluster variability. This agrees with the reduction of DMI when the proportion of concentrates in the diet was increased.⁴⁴ From these, fattening duration, which refers to the growing of animals to a desired end-point can be further used as a lever proxy to achieve/manage the desirable quality of carcass or meat. Indeed, we have previously validated the fattening period duration as a proxy of fat tissue and muscle per cents⁴³ and as a predictor of beef tenderness or flavor classes of young bulls^{5, 11, 45}.

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

Overall, to achieve the desirable fatness score and thus tender beef, the young bulls must be more efficient under accurate feeding and duration period. The findings emphasized that the oldest and heaviest animals fattened for a long period with forage are not likely to give tender meat but lead to better carcass properties *i.e.*, the heaviest with high conformation scores and 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.

References

1. Miller MF, Carr MA, Ramsey CB, Crockett KL and Hoover LC, Consumer thresholds for establishing the value of beef tenderness. *J Anim Sci* **79**:3062-3068 (2001).
2. Boleman SJ, Boleman SL, Miller RK, Taylor JF, Cross HR, Wheeler TL, Koohmaraie M, Shackelford SD, Miller MF, West RL, Johnson DD and Savell JW, Consumer evaluation of beef of known categories of tenderness. *J Anim Sci* **75**:1521-1524 (1997).
3. O'Quinn TG, Legako JF, Brooks JC and Miller MF, Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience¹. *Translational Animal Science* **2**:26-36 (2018).
4. Monteils V, Sibra C, Ellies-Oury MP, Botreau R, De la Torre A and Laurent C, A set of indicators to better characterize beef carcasses at the slaughterhouse level in addition to the EUROP system. *Livestock Science* **202**:44-51 (2017).
5. Gagaoua M, Picard B, Soulat J and Monteils V, Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. *Livestock Science* **214**:245-258 (2018).
6. Gagaoua M, Monteils V, Couvreur S and Picard B, Identification of Biomarkers Associated with the Rearing Practices, Carcass Characteristics, and Beef Quality: An Integrative Approach. *J Agric Food Chem* **65**:8264-8278 (2017).
7. Devlin DJ, Gault NFS, Moss BW, Tollard E, Tollerton J, Farmer LJ and Gordon AW, Factors affecting eating quality of beef. *Advances in Animal Biosciences* **8**:s2-s5 (2017).

8. Hocquette JF, Van Wezemael L, Chriki S, Legrand I, Verbeke W, Farmer L, Scollan ND, Polkinghorne R, Rodbotten R, Allen P and Pethick DW, Modelling of beef sensory quality for a better prediction of palatability. *Meat science* **97**:316-322 (2014).
9. Dunshea FR, D'Souza DN, Pethick DW, Harper GS and Warner RD, Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat science* **71**:8-38 (2005).
10. Soulat J, Picard B, Léger S and Monteils V, Prediction of beef carcass and meat quality traits from factors characterising the rearing management system applied during the whole life of heifers. *Meat science* **140**:88-100 (2018).
11. Gagaoua M, Monteils V and Picard B, Decision tree, a learning tool for the prediction of beef tenderness using rearing factors and carcass characteristics. *J Sci Food Agric* **99**:1275-1283 (2019).
12. Warner RD, Greenwood PL, Pethick DW and Ferguson DM, Genetic and environmental effects on meat quality. *Meat science* **86**:171-183 (2010).
13. Hocquette JF, Botreau R, Picard B, Jacquet A, Pethick DW and Scollan ND, Opportunities for predicting and manipulating beef quality. *Meat science* **92**:197-209 (2012).
14. Gagaoua M, Terlouw EMC, Micol D, Hocquette JF, Moloney AP, Nuernberg K, Bauchart D, Boudjellal A, Scollan ND, Richardson RI and Picard B, Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. *Journal of Integrative Agriculture* **15**:1550-1563 (2016).
15. Listrat A, Lebret B, Louveau I, Astruc T, Bonnet M, Lefaucheur L, Picard B and Bugeon J, How Muscle Structure and Composition Influence Meat and Flesh Quality. *TheScientificWorldJournal* **2016**:3182746 (2016).
16. Renand G, Jurie C, Robelin J, Picard B, Geay Y and Ménissier F, Genetic variability of muscle biological characteristics of young Limousin bulls. *Genetics Selection Evolution* **27**:287 (1995).
17. Gregory KE, Cundiff LV and Koch RM, Genetic and phenotypic (co)variances for growth and carcass traits of purebred and composite populations of beef cattle. *J Anim Sci* **73**:1920-1926 (1995).
18. Gagaoua M, Picard B and Monteils V, Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. *Meat science* **140**:145-156 (2018).
19. Dubost A, Micol D, Meunier B, Lethias C and Listrat A, Relationships between structural characteristics of bovine intramuscular connective tissue assessed by image analysis and collagen and proteoglycan content. *Meat science* **93**:378-386 (2013).
20. Hill F, The Solubility of Intramuscular Collagen in Meat Animals of Various Ages. *Journal of Food Science* **31**:161-166 (1966).
21. Folch J, Lees M and Sloane Stanley GH, A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of biological chemistry* **226**:497-509 (1957).
22. Gagaoua M, Micol D, Picard B, Terlouw CE, Moloney AP, Juin H, Météau K, Scollan N, Richardson I and Hocquette JF, Inter-laboratory assessment by trained panelists from France and the United Kingdom of beef cooked at two different end-point temperatures. *Meat science* **122**:90-96 (2016).
23. Young G and Householder AS, Discussion of a set of points in terms of their mutual distances. *Psychometrika* **3**:19-22 (1938).
24. Næs T, Brockhoff PB and Tomic O, Cluster Analysis: Unsupervised Classification, in *Statistics for Sensory and Consumer Science*, ed. by Næs T, Brockhoff PB and Tomic O (2010).
25. Koknaroglu H, Loy DD, Wilson DE, Hoffman MP and Lawrence JD, Factors Affecting Beef Cattle Performance and Profitability. *The Professional Animal Scientist* **21**:286-296 (2005).

26. Lawrence T, Fowler V and Novakofski J, *Growth of Farm Animals: 3rd edition*. Cabi (2012).
27. Jurie C, Martin J-F, Listrat A, Jailler R, Culioli J and Picard B, Effects of age and breed of beef bulls on growth parameters, carcass and muscle characteristics. *Animal Science* **80**:257-263 (2005).
28. Shorthose WR and Harris PV, Effect of Animal Age on the Tenderness of Selected Beef Muscles. *Journal of Food Science* **55**:1-+ (1990).
29. Shackelford SD, Koohmaraie M and Wheeler TL, Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. *Journal of Animal Science* **73**:3304-3309 (1995).
30. Wulf DM, Morgan JB, Tatum JD and Smith GC, Effects of animal age, marbling score, calpastatin activity, subprimal cut, calcium injection, and degree of doneness on the palatability of steaks from limousin steers. *J Anim Sci* **74**:569-576 (1996).
31. Purslow PP, New developments on the role of intramuscular connective tissue in meat toughness. *Annu Rev Food Sci Technol* **5**:133-153 (2014).
32. Chriki S, Gardner GE, Jurie C, Picard B, Micol D, Brun JP, Journaux L and Hocquette JF, Cluster analysis application identifies muscle characteristics of importance for beef tenderness. *BMC Biochem* **13**:29 (2012).
33. Maltin CA, Sinclair KD, Warriss PD, Grant CM, Porter AD, Delday MI and Warkup CC, The effects of age at slaughter, genotype and finishing system on the biochemical properties, muscle fibre type characteristics and eating quality of bull beef from suckled calves. *Animal Science* **66**:341-348 (1998).
34. Keane MG, Beef production from Holstein–Friesian bulls and steers of New Zealand and European/American descent, and Belgian Blue×Holstein–Friesians, slaughtered at two weights. *Livestock Production Science* **84**:207-218 (2003).
35. Steen RWJ and Kilpatrick DJ, Effects of plane of nutrition and slaughter weight on the carcass composition of serially slaughtered bulls, steers and heifers of three breed crosses. *Livestock Production Science* **43**:205-213 (1995).
36. Moloney AP and McGee M, Chapter 2 - Factors Influencing the Growth of Meat Animals, in *Lawrie's Meat Science (Eight Edition)*. Woodhead Publishing, pp 19-47 (2017).
37. Owens FN, Gill DR, Secrist DS and Coleman SW, Review of some aspects of growth and development of feedlot cattle. *J Anim Sci* **73**:3152-3172 (1995).
38. Muir PD, Deaker JM and Bown MD, Effects of forage- and grain-based feeding systems on beef quality: A review. *New Zealand Journal of Agricultural Research* **41**:623-635 (1998).
39. Al-Jammas M, Agabriel J, Vernet J and Ortigues-Marty I, The chemical composition of carcasses can be predicted from proxy traits in finishing male beef cattle: A meta-analysis. *Meat science* **119**:174-184 (2016).
40. Keane MG, Drennan MJ and Moloney AP, Comparison of supplementary concentrate levels with grass silage, separate or total mixed ration feeding, and duration of finishing in beef steers. *Livestock Science* **103**:169-180 (2006).
41. Franco D, Bispo E, Gonzalez L, Vazquez JA and Moreno T, Effect of finishing and ageing time on quality attributes of loin from the meat of Holstein-Friesian cull cows. *Meat science* **83**:484-491 (2009).
42. Juniper DT, Browne EM, Fisher AV, Bryant MJ, Nute GR and Beever DE, Intake, growth and meat quality of steers given diets based on varying proportions of maize silage and grass silage. *Animal Science* **81**:159-170 (2007).
43. Soulat J, Picard B, Leger S and Monteils V, Prediction of beef carcass and meat traits from rearing factors in young bulls and cull cows. *J Anim Sci* **94**:1712-1726 (2016).

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44. Steen RWJ and Kilpatrick DJ, The effects of the ratio of grass silage to concentrates in the diet and restricted dry matter intake on the performance and carcass composition of beef cattle. *Livestock Production Science* **62**:181-192 (2000).

45. Gagaoua M, Picard B and Monteils V, The farm-to-table continuum as an innovative trade-off approach for optimal management decisions in the beef sector: flavour clustering of young bull meat cuts, in *10 International Symposium on the Nutrition of Herbivores (ISNH10)*, Ed. Cambridge University Press, Clermont-Ferrand, pp 143-143 (2018).

46. Herva T, Huuskonen A, Virtala A-M and Peltoniemi O, On-farm welfare and carcass fat score of bulls at slaughter. *Livestock Science* **138**:159-166 (2011).

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.

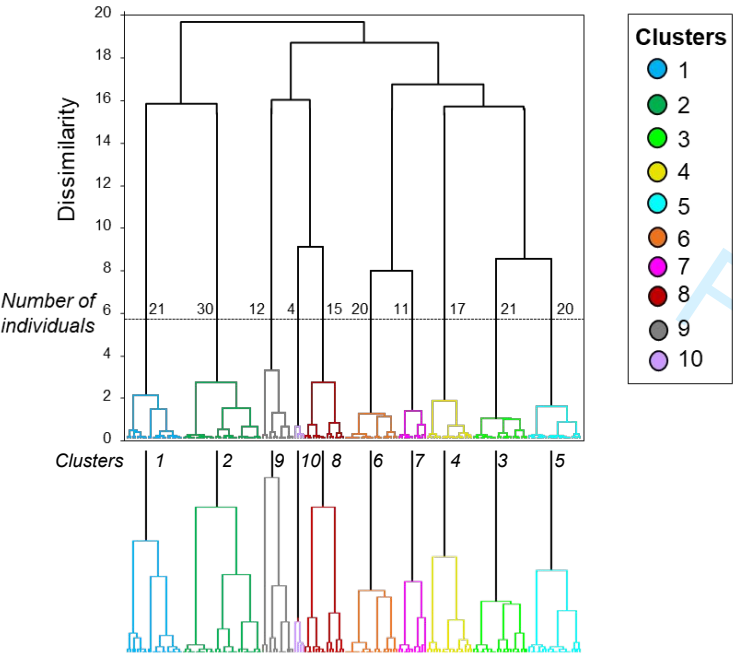
Figure 3. Correlation charts showing the individual cluster variability and associations between mean tenderness scores and rearing factors per cluster. Each point represents the mean value of each animal cluster and the error bars the individual's variability. Significant correlations by their R^2 ($P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

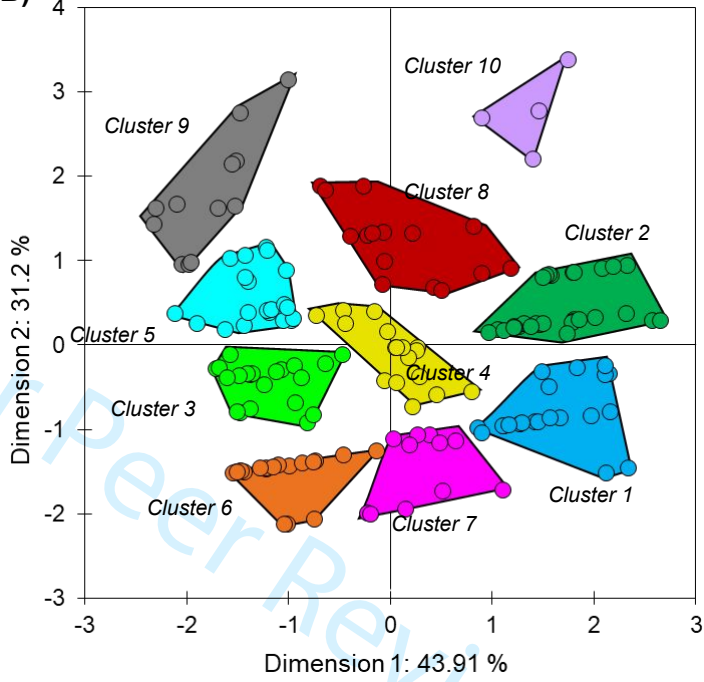
Figure 5. Correlation charts showing the individual cluster variability and associations between mean tenderness scores and muscle characteristics and meat quality traits per cluster. Each point represents the mean value of each animal cluster and the error bars the individual's variability. Significant 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)

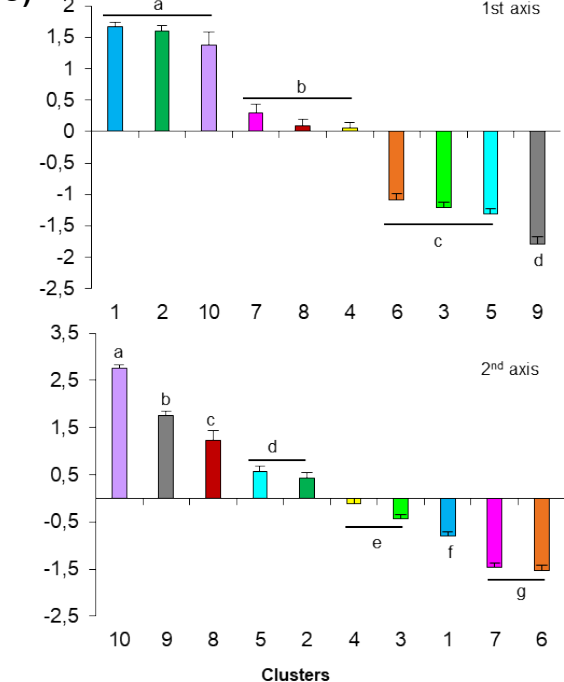


Figure 1.

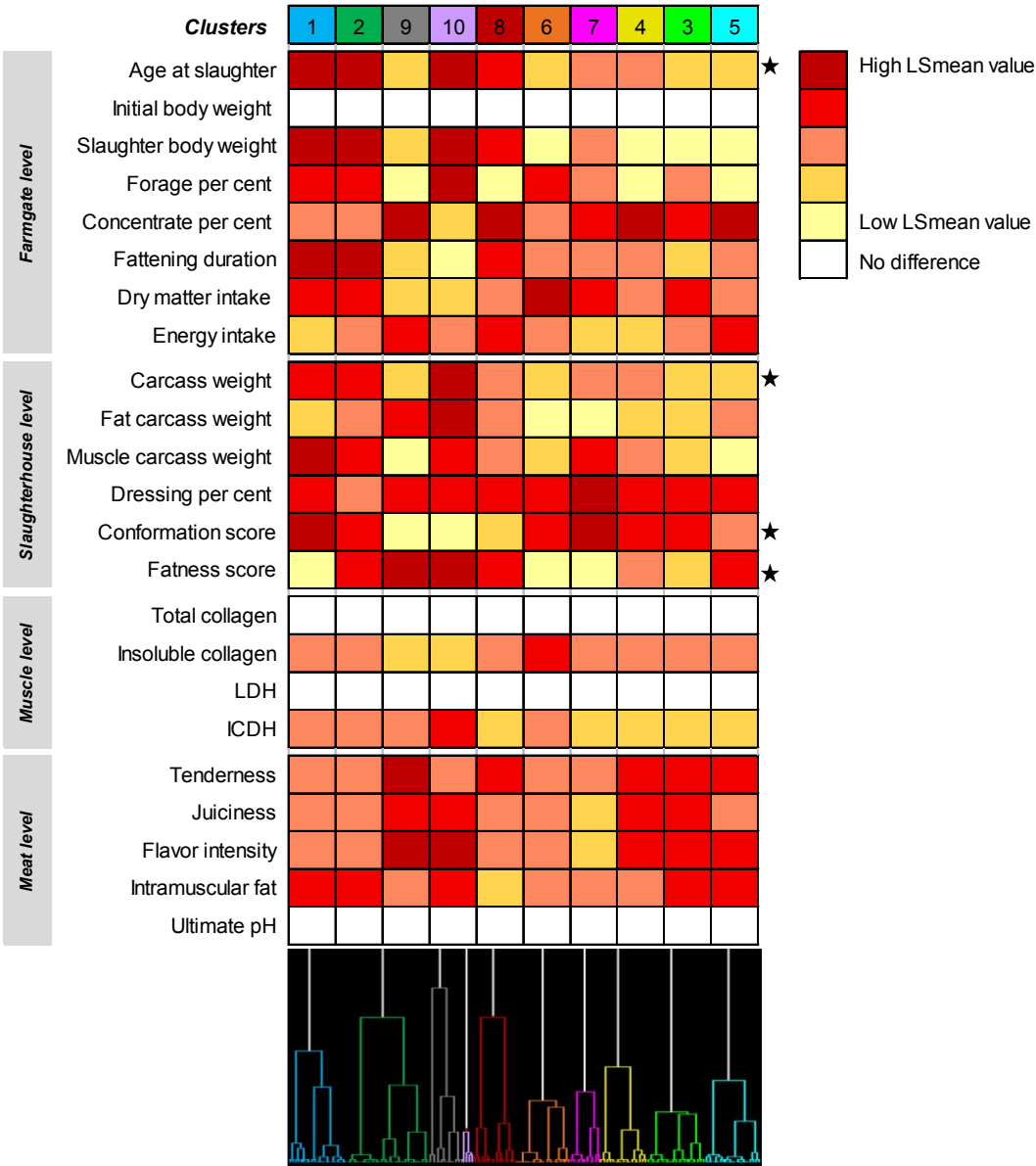


Figure 2.

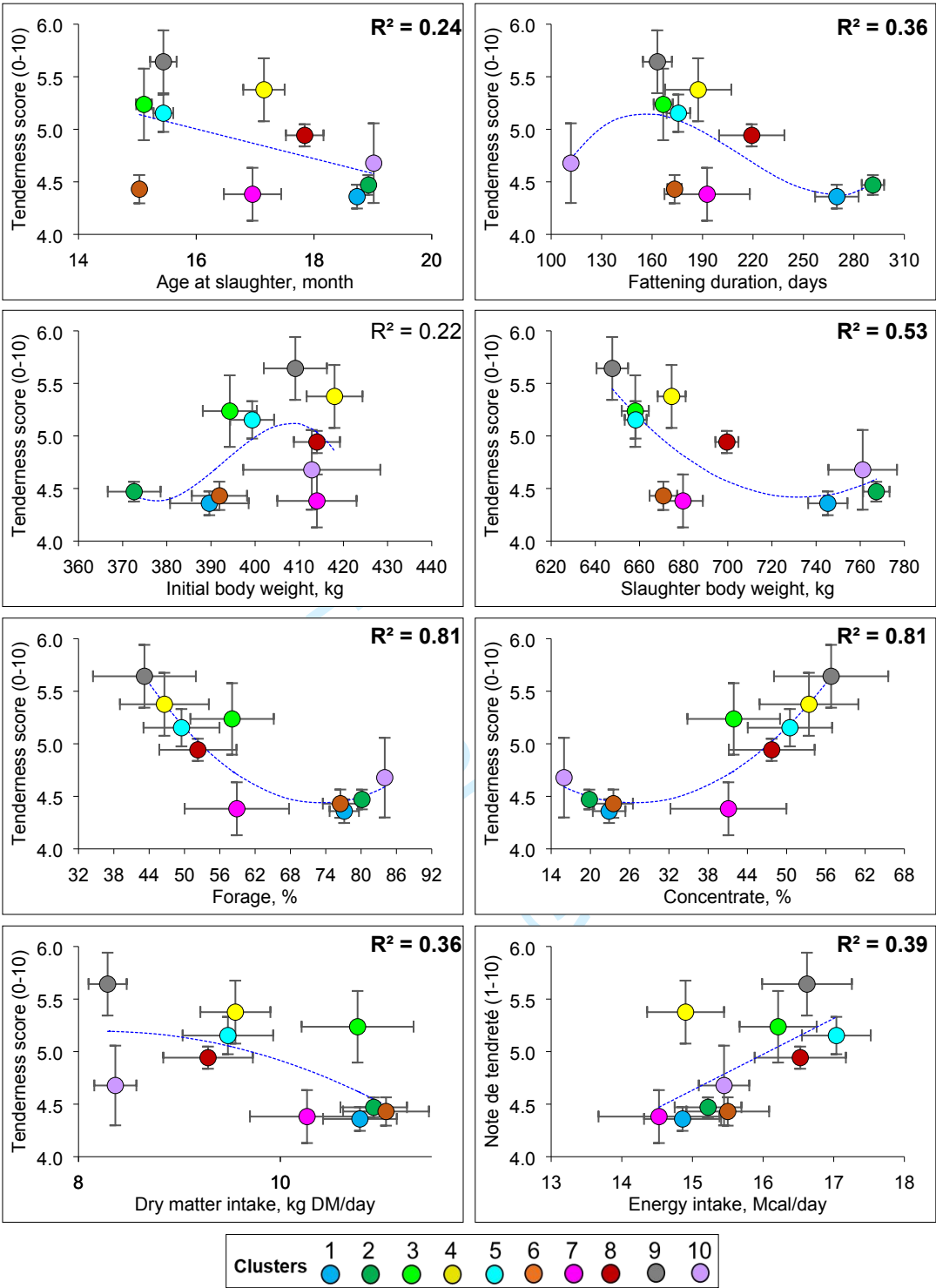


Figure 3.

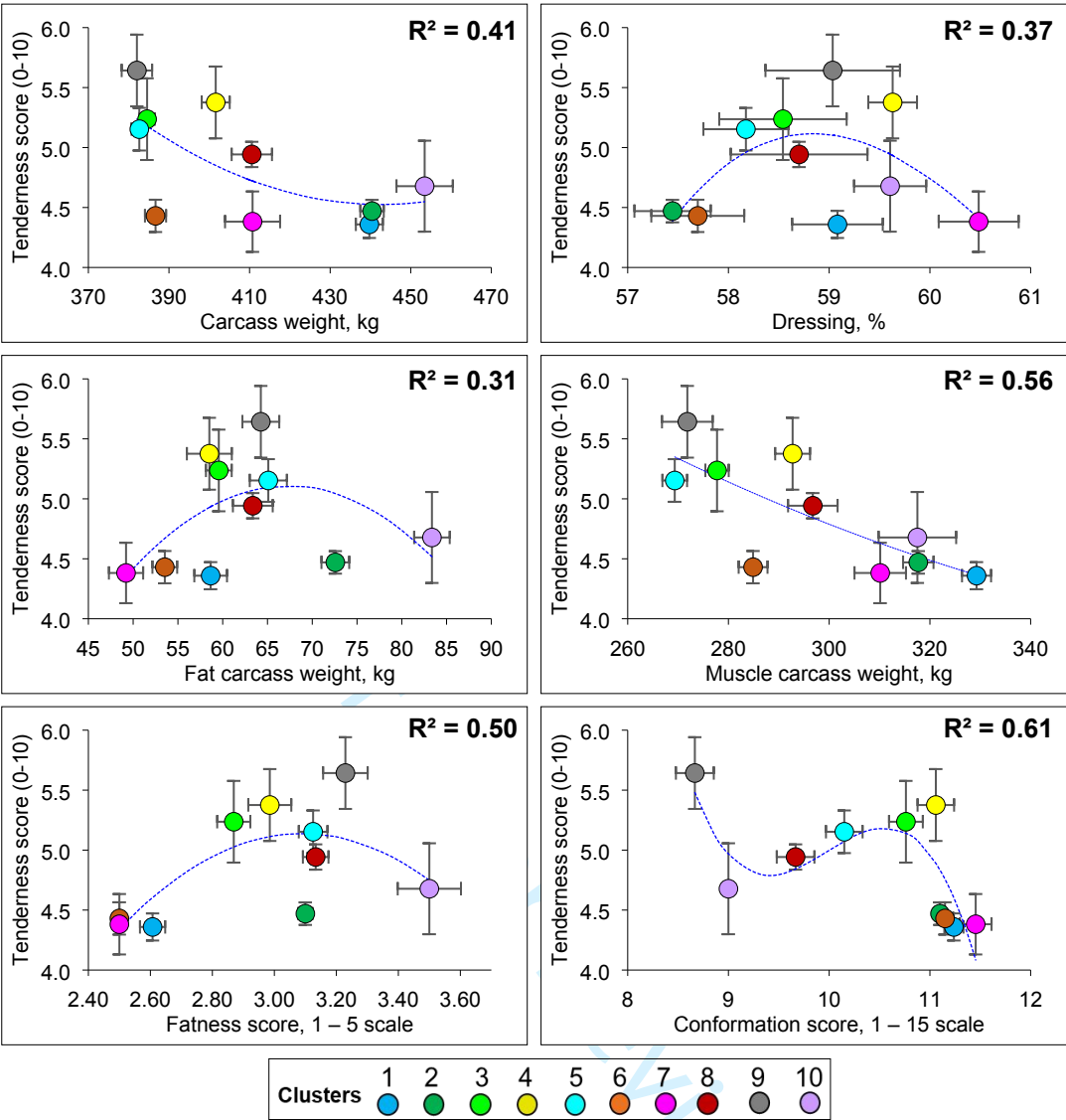


Figure 4.

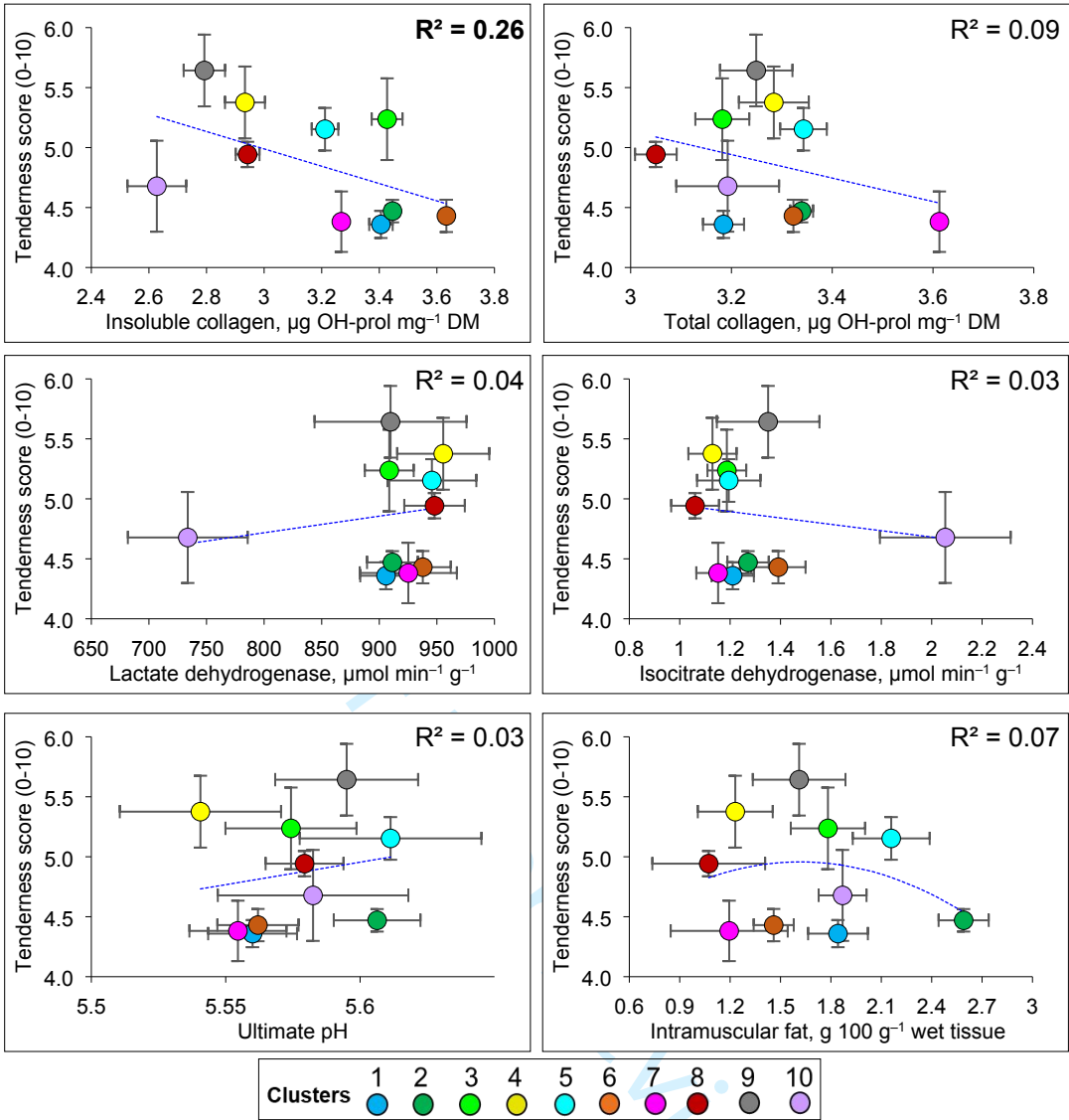


Figure 5.



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.

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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	<i>n</i> =	Clusters										SEM	<i>P</i> -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	<i>n</i> =	Clusters										SEM	<i>P-value</i>
		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, µg OH-prol mg ⁻¹ 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, µg OH-prol mg ⁻¹ 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), µmol min ⁻¹ 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), µmol min ⁻¹ 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; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

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

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Appendices

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.

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