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Clustering of sensory eating qualities of beef: consistencies and differences within carcass, muscle, animal characteristics and rearing factors

Mohammed Gagaoua*, Brigitte Picard, Julien Soulat, Valérie Monteils**

Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

Correspondence to: Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

E-mail adresses:

- * Dr. Mohammed Gagaoua: gmber2001@yahoo.fr; mohammed.gagaoua@inra.fr;
- ** Dr. Valérie Monteils: valerie.monteils@vetagro-sup.fr

Abstract

pHu and palatability traits (tenderness, juiciness and beef flavor intensity) of 480 young bulls were investigated. For each trait, quality categories were built using unsupervised learning methods to relate them with the farm-to-table continuum data. The categorization into high, medium and low-quality clusters showed differences at the whole levels of the continuum including animal and rearing factors, carcass, muscle and meat parameters. Isocitrate dehydrogenase activity, weight at the beginning of the fattening period and fattening duration were robust discriminators within the clusters. When focused on palatability traits, the different estimates of fat (%fat carcass, fat carcass weight of the 6th rib, fatness score and intramuscular fat) and %muscle carcass allow the categorization of the traits into classes. Furthermore, the tender, juicy and flavorful meat had the lowest total collagen content as the corresponding animals fed with the lowest %forage and dry matter intake. The findings evidenced the potential of the different levels of the farm-to-table continuum to achieve the requested quality.

Keywords: Young bulls; Sensory meat quality; Carcass properties; Machine learning tools; Farm-to-table continuum.

1. Introduction

After animal bleeding, the muscle tissue is converted into meat during the *post-mortem* period (<u>Ouali et al., 2013</u>). During this period, a complex cascade of energetic, biochemical, and physical changes take place in the muscle and lead to the development of the meat palatability, like tenderness, juiciness and flavor (<u>Savell and Cross, 1988; Listrat et al., 2016; Flores, 2017</u>). Despite considerable efforts to improve beef eating qualities, research has shown that the determinism behind this sophisticated mechanism involving myriad biological pathways, including pH decline (<u>Ouali et al., 2013; Lomiwes et al., 2014; Gagaoua et al., 2015; Gagaoua et al., 2016b; Listrat et al., 2016</u>), is not fully understood and a great variability in the meat quality traits steels uncontrolled.

The main factors that were described over decades to affect beef eating qualities are both intrinsic (breed, sex, age, inter-animal variability, contractile and metabolic muscle properties) and extrinsic (season, feeding, rearing factors, hormones, transport, animal handling and post-slaughter ageing conditions) (Ferguson et al., 2001; Hocquette et al., 2012; Gagaoua et al., 2016b; Listrat et al., 2016). However, the factors were mostly investigated objectively using different breeds and animal's types, depending on the final goal of each trial. This may be the reason, which may explain the inconsistencies between experiments. Therefore, providing a powerful system that would help for better management and prediction of the potential of carcasses and beef eating qualities is the priority of the stakeholders. Accordingly, several carcass and meat quality grading schemes were described in different countries. To date, three systems in three continents were used for a large proportion of beef production and processing (Dikeman, 2017), namely the EUROP system for the European countries, the USDA beef carcass grading system in the United States and the Meat Standards Australia (MSA) system in Australia. However, these three systems are different and the rearing management systems applied by farmers are not already considered. For example, the EUROP system needs to include further additional indicators for better carcass grading (Monteils et al., 2017). Eating quality is not yet part of this last grading system. The USDA and MSA systems consider the eating quality but only very few animal traits or rearing factors. Meanwhile, considering the entire life of the animals and more specifically the fattening period factors would be useful for joint management of carcass and meat qualities (Oury et al., 2007; Soulat et al., 2016; Gagaoua et al., 2017b).

Thus, implementation of a holistic study by considering the farm-to-fork live period of the animals is a challenging task and would characterize adequately and sufficiently the factors in relation to the desirable qualities. To our knowledge, there is scarcity in the published works that have studied the meat and carcass qualities basis under the continuum rearing practices – final qualities of beef carcasses. This study deals with these aspects and intends to consider the farm-to-table continuum by using unsupervised learning and clustering methods for optimal management decisions in the beef sector. Hence, for instance the first objective is to consider the variability between 480 French young bulls by their categorization into sensory eating qualities clusters (classes) and then to relate them with animal, rearing factors, carcass and muscle data. Furthermore, the aim is to understand the multi-dimensional nature of the factors that would discriminate between different quality trait-clusters. The clustering approach would provide the rearing factors and animal traits, as well as live-animal performances, carcass and muscle characteristics that need improvement to reach the targeted market specifications for both stakeholders and consumers.

2. Materials and Methods

2.1. Animals, handling and slaughtering

The study was carried out as part of a research program approved by the Ethical Committee of INRA Auvergne-Rhône-Alpes (ARA) (National Institute for Agricultural Research, INRA-Herbipôle, Theix, France). Individual data from 6 experiments grouping a total of 480 young bulls were used in this study. The dataset included Charolais (n = 264), Salers (n = 93), Limousin (n = 74), and Blond d'Aquitaine (n = 49) breeds. The 4 breeds are the major suckled breeds slaughtered in France. For the Charolais breed dataset, the animals are from 5 consecutive years' experiments most of the from the experimental unit INRA-UE232 (Bourges). All the animals and irrespective of experiment and production year were raised and managed under similar practices in the INRA research center. The animals had, ad libitum, the similar ration 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. Diets were formulated according to the recommendations of INRA (2007) to meet NEg requirements needed for maintenance and theoretical body weight gain. The young bulls were handled in accordance with the French animal protection regulations defined in the French law (Code Rural, articles R214-64 to R214-71; Legifrance, 2016). Before slaughter, all animals were fasted for 24 h and had free access to water. The animals

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 as is usual in most experimental and industrial French slaughterhouses and they were stored at a maximum temperature of 4°C (between 2 and 4°C) until 24 h *post-mortem* (*p-m*) according to standard commercial practice.

2.2. Muscle sampling

Muscle samples of *Longissimus thoracis* (LT, mixed fast oxido-glycolytic muscle) were excised between the sixth and eleventh ribs from the right-hand side of the carcass of each animal 24 h *p-m*. The epimysium was carefully dissected and four sub-samples were used. The first one (approximately 5 g) was subsequently frozen in liquid nitrogen and kept at – 80°C until analyzed for the determination of enzyme activities, protein extraction and fiber area quantification. The second part was cut into pieces (1–2 cm cross-section), vacuum packed and stored at –20 °C until analyzed for collagen and intramuscular fat content. The third part was used for meat color measurement. The last part of the samples for sensory evaluation were cut into steaks (20 mm thick), placed in sealed plastic bags under vacuum and kept at 4°C for ageing (14 days). Each LT sample was then frozen and stored at –20°C until sensory evaluation and shear force measurements. All evaluations of muscle characteristics were conducted by the same laboratory.

2.3. Rearing factors characterizing the fattening period

The rearing practices of the 480 young bulls were characterized by 12 rearing factors similar to those described by (Soulat et al., 2016). They include slaughter age (months), fattening duration (days), initial body weight (initial BW, kg) at the beginning of the fattening period, final BW (kg), dry matter intake (DMI, kg DM/day), total forage and concentrate intakes (kg DM/day), forage and concentrate in percentage (in the DM diet), energy intake (Mcal/day), average daily gain (ADG) for the fattening period (kg/day), and feed efficiency (ADG/DMI ratio, kg/kg DM).

2.4. Carcass traits

Subsequently, the carcasses were graded under the EU beef carcass classification (EUROP) scheme. This was done at the slaughterhouse using experts familiar with the

EUROP grid (Commission Regulation (EC) 1249/2008). Under EUROP grid, five conformation classes are 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, 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). At the same time, the degree of fat cover of the carcasses (hereafter fatness score) which describes the amount of fat on the outside of the carcass was numerically scored from 1 = leanest to 5 = fattest.

2.5. Carcass composition by the 6th rib dissection

Each carcass was characterized for hot carcass weight and thus for cold carcass weight. The carcass weight is typically measured within 2 h of slaughter and is the weight of the animal after the removal of its head, hide, feet/legs, thoracic organs, internal fats, and abdominal organs and is expressed as cold carcass weight, which is 0.98 times the hot carcass weight according to the EUROP classification guidelines (Commission Regulation (EC) 1249/2008). The carcass weight was used to calculate the dressing% and carcass composition. For carcass composition, the % of muscle carcass weight, % of fat tissue carcass weight, and % of bone carcass weight were computed. For that, the entire 6th rib at the margins of the 5th and 7th ribs was excised from the right side of each carcass and weighed. The whole carcass composition was estimated from the composition of the 6th rib determined after physical dissection following standard commercial practice involving a close trimming and deboning of the muscle (Gagaoua et al., 2018b). The physical dissection of the ribs into fat, muscle and bone is the reference method to assess the composition of carcasses (Robelin and Geay, 1975). The total dissected weights of muscle, bone and fat were recorded by this method. After that, prediction equations were used to compute the different characteristics after slight adjustments of the equation models at the internal INRA research center slaughterhouse (Robelin and Geay, 1975).

2.6. Muscle characteristics determination

For metabolic muscle type determination, the measurements of glycolytic and oxidative enzyme activities of respectively lactate dehydrogenase (LDH; EC 1.1.1.27) and isocitrate dehydrogenase (ICDH; EC 1.1.1.42) were performed (Ansay et al., 1974; Briand et al., 1981).

These enzymes are representative of different steps of the glycolytic and oxidative pathways that are currently used for the characterization of the metabolic types of beef muscles (Gagaoua et al., 2016b).

For fiber surface quantification, the samples were taken from the freezer, transported in liquid nitrogen and thin transverse serial sections (10 μ m thick) were sliced at – 20°C using a cryostat (Cryo-star HM 560 Microm international GmbH, Germany). For the cutting, the muscle samples were perpendicularly fixed in the metallic supports of the microtome embedded in Tissue-Tek® (O.C.T. Compound), to obtain several series of cross-sections that are then fixed in previously identified slides. Muscle fiber orientation and sample integrity were verified in the first cross-section series through microscopy. The slides were analyzed under a Nikon microscope, randomly selecting two fields that had been individually captured using a Visilog image analysis system. Mean cross sectional area (μ m²) was determined, based on 100 to 200 fibers, regardless of contractile or metabolic fiber type.

For collagen determination, frozen muscle was homogenized in a household cutter, freezedried for 48h and pulverized in a horizontal blade mill. To estimate total and insoluble collagen, the amount of total hydroxyproline (OH-prol) content from the lyophilized muscle powder was measured according to the protocol described by (<u>Dubost et al., 2013</u>). Briefly, 250 mg of muscle powder were weighed, acid hydrolyzed with 10 mL of 6 N HCl overnight at 105°C in a screw-capped glass tube. For total collagen, the acid hydrolysate was diluted 5 times in water. Heat soluble collagen was extracted after heating lyophilized muscle powder with a buffer containing 0.23 M NaCl, 25 mM Tris–HCl, (pH 7.4) at 75 °C for 1 h with stirring every 15 min. The homogenates were centrifuged at 5000 rpm and the supernatants were discarded. The pellets were hydrolyzed following the procedure used for total collagen. For both total and insoluble collagen, each sample was weighed and measured in triplicate.

For intramuscular fat (IMF) content, 6 g of lyophilized muscle powder were mixed with chloroform-methanol for their extraction and assayed gravimetrically (<u>Gagaoua et al., 2016b</u>). Each sample was measured in triplicate and muscle total lipids are reported as g of fatty acid per 100 g wet tissue.

2.7. Meat quality evaluation

For muscle pH at 24h *post-mortem*, the measurements were conducted by inserting a glass electrode on the LT muscle between the 6th and 7th rib using a Hanna HI 9025 pH/ORP meter suitable for meat penetration (Hanna Instruments Inc., Woonsocket, RI, USA). Before

measurement, the pH meter was first calibrated at chilling temperature using pH 4 and pH 7 buffers.

For determination of the initial meat color (not color stability), a Minolta colorimeter (Minolta CR400, Konica Minolta, Japan) was used to measure objective meat color using illuminant D65, 8 mm diameter aperture and 10° standard observer (Gagaoua et al., 2018b). The colorimeter was calibrated prior to color determination using standard white tiles (Y = 93.58, x = 0.3150, and y = 0.3217). Fresh ribeye cut slices of not less than 2.5 cm thick and overwrapped with highly plasticized polyvinyl chloride [0.13 mm thick, oxygen transmission rate (OTR) = 22,500 cm³/m²/24 h at 23°C, water vapor transmission rate = 496 g/ m²/24 h at 37.8°C and 90% relative humidity] were left on a polystyrene tray to refrigerate at 4°C for 1h to allow blooming. Color coordinates were measured in the CIE color convention (CIE, 1986) where the three fundamental outputs are L^* , a^* and b^* coordinates. L^* is the lightness on a scale of 0 (black) to 100 (white); a^* spans from +60 (red) to -60 (green) and b^* spans from +60 (yellow) to -60 (blue). Afterwards, mathematical operations were used to calculate the parameters Chroma (C^*), related to the intensity of color (higher when a^* of b^* are high), and hue angle (h^*), related to the change of color from red to yellow, using the following equations: $C^* = [(a^{*2} + b^{*2})^{1/2}]$ and $h^* = [(b^*/a^*) \tan^{-1}]$.

For sensory meat assessment, steaks aged for 14 days were thawed, without stacking or overlapping, at 5°C in vacuum packs for 48 h before cooking and sensory assessment at the usually used end-point temperature in France, 55 °C (Gagaoua et al., 2016a). One hour before sensory assessment, the meat samples were cut into approximately 1.50 cm thick steaks and grilled on a double grooved plate griddle (SOFRACA, Morangis, France) heated to 300°C for 30 min before cooking. Steaks were heated for 2 min until the end-points temperature of 55°C in the geometric center of the steak was reached using a temperature probe (Type K, HANNA HI 98704, Newark, USA). After grilling, each steak was cut into 20 mm cubes that were immediately served to 12 panelists chosen according to the criteria described by (Gagaoua et al., 2016a). The panelists rated the steaks on a 10-cm unstructured line scale (from 0 to 10) measured in mm for the following sensory attributes:

Global tenderness defined as the ease of chewing the sample between teeth: from extremely tough (0) to extremely tender (10).

Juiciness defined as the amount of moisture released in the mouth: from extremely dry (0) to extremely juicy (10).

Beef flavor defined as the flavor associated with cooked beef: extremely weak beef flavor (0) to extremely strong beef flavor (10)

The sessions were carried out in a sensory analysis room equipped with individual booths under artificial red light to reduce the influence of the appearance of the samples. At each session, a monadic presentation of 6 samples was done, each sample being selected in random order. 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, which were later formatted in Excel.

2.8. Statistical analyses

The individual data were analyzed using SAS statistical software (SAS 9.4, SAS Institute INC, Cary, NC, USA) and XLSTAT 2017.19.6 (AddinSoft, Paris, France). Before analysis, data exploration and database preparation were scrutinized for data entry errors and outliers using Smirnov–Grubb's outlier test at 5% level. After this, a general description analysis was performed by computing size, mean, standard deviation and minimum and maximum ranges of each variable (**Table 1**).

For animal clustering into meat eating quality clusters, unsupervised learning methods (*k*-means, univariate partitioning and hierarchical cluster analysis (HCA) were tested and those retained because they gave the best results were *k*-means algorithm and HCA. The former is an iterative clustering method used to automatically partition a dataset into *k* groups where the number of clusters *k* is assumed to be fixed *a priori* by minimizing relative distances and the latter is a cluster analysis method, which seeks to build a hierarchy and binary tree of clusters. Before clustering, for each quality trait cluster (pHu, tenderness, juiciness and beef flavor), the missing data were considered as missing at random regarding the outcome variable. It was thus decided to exclude cattle with missing data to construct the meat eating quality clusters and not impute missing data values (Donders et al., 2006). Moreover, for each quality trait and before clustering, the data were first normalized for each confounded or present effect in the database by computing Z-scores. Z-scores represent the deviation of each trait observation relative to the mean of the corresponding animal irrespective of the breed, experiment and other confounded effects and were calculated using PROC STANDARD of SAS, which standardizes data to a mean of 0 and standard deviation of 1.

The average silhouette width (S_i) criterion was used for the validation of the clustering results (*i.e.* to choose the best clustering method and number of clusters) as it is based on the proximity among objects of a certain cluster, and the proximity of these objects to the nearest

clusters. Silhouette width refers to a succinct graphical representation method for interpretation and validation of consistency within clusters of data. It is a measure of how similar an object is to its own cluster (cohesion) compared to other clusters (separation) (Rousseeuw, 1987). The value of silhouette width ranges from -1 to +1, and can be interpreted as follow: i) observations with a large S_i (almost 1) are very well clustered; ii) a small S_i (around 0) means that the observation lies between two clusters and iii) observations with a negative S_i are probably placed in the wrong cluster. S_i can be computed with any distance metric, such as the Euclidean distance or the Manhattan distance. For the significance of differences between the least squares means of the variables between the clusters, the Proc GLM of SAS was performed. For comparisons, the Tukey's test at a significance level of P < 0.05 was used.

3. Results and discussion

The description of the 39 variables corresponding to carcass characteristics, eating quality traits, muscle characteristics, animal characteristics and rearing factors evaluated for the French young bulls are given in **Table 1**. From these data, the beef quality traits, namely ultimate pH, tenderness, juiciness and beef flavor were used to build clusters that are presented and discussed in the following sections.

3.1. Ultimate pH clusters

Prior to the clustering of the animals according to LT muscle pHu-values, Grubb's test at a significance level of $\alpha = 0.05$ was first used to identify extreme values. Within the 377 data of pHu, the detected extreme values account for 9 animals with values > 6.0 (mean of 6.16 \pm 0.12 [6.0 – 6.36]). These 9 animals would highlight the dark-cutting defect with an incidence in this trial of pHu > 6.0 of 2.4%. In France, it has been reported that the proportion of LT muscle of bulls with pHu values greater than 6.0, was 3.36% (Mounier et al., 2006) and for different sexes (young bulls, steers and cows) of purebred Charolais cattle was 0.11% (Gagaoua et al., 2018b). In a Spanish study, this incidence was 4.02% (Mach et al., 2008). However, the pHu-means of the present study were within the normal range for cattle (Jeremiah et al., 1991; Gagaoua et al., 2016b; Gagaoua et al., 2017a; Gagaoua et al., 2018b) as the average values were 5.59 \pm 0.14 and 5.57 \pm 0.11 with or without the extreme values, respectively. This indicates for most of the animals, *i.e.*, 97.6% of the young bulls that had final pH below 6.0, did not experience pre-slaughter stress known to be related with glycogen loss.

The primary k-means and HCA clustering analyses allowed the identification of 4 clusters of pH (data not shown). The 4th cluster from both analyses grouped the same 9 extreme pHu-values. From these results together to those of Grubb's test, another clustering was performed by removing the 9 animals with extreme pHu-values. Three clusters were then produced (**Table 2**) and those of HCA were validated based on the best silhouette widths ($S_i = 0.59$ by HCA vs. 0.45 by k-means). The lowest pHu cluster [pH_u-, grouped 145 animals (39.41%)] had an average value of 5.48 (±0.06). The medium pHu cluster [pH_u-, grouped 144 animals (39.13%)] had an average value of 5.59 (±0.03). The high pHu cluster [pH_u+, grouped 79 animals (21.46%)] had an average value of 5.70 (±0.08). The differences between clusters for carcass characteristics, meat qualities, muscle characteristics and animal characteristics and rearing factors are summarized in **Table 2**.

3.1.1. Carcass and muscle characteristics

For carcass characteristics, the carcass and muscle carcass weights were the only two variables from the 12 studied to differ significantly (P < 0.05) between pH_u-clusters. They were both the highest in pH_u- and pH_u= compared to pH_u+ cluster. The carcass weight is known to affect the final muscle pH (McGilchrist et al., 2012). This is likely to have been due to the rate of pH decline as it is related to myosin isoform expression, a consequence of muscle growth response (Pike et al., 1993) or protein turnover (Oksbjerg and Therkildsen, 2017). Accordingly, we have previously reported that the animals with greater growth rates also have muscles containing greater proportion of glycolytic muscle fibers (Duris et al., 1999) known to link with the sudden decline of carcass pH (Gardner et al., 2006). In this study, this was supported by the high and low activities of the enzymes involved in the oxidative and the glycolytic pathways respectively found in the pH_u+ cluster (**Table 2**). We suppose also that the link between carcass weight and pH decline would reflect muscle glycogen levels and carcass temperature interactions as extensively discussed in a comprehensive review by (Jacob and Hopkins, 2014).

A confounded effect with other factors, likely age at slaughter (Jurie et al., 2005) and further rearing factors (see below) might be also at the origin of these differences. However, the difference in final pH between the clusters was small (<0.22 pH units) and the narrow pHu mean values of this study were within the normal and acceptable range (*i.e.* 5.4 to 5.8) for beef cuts (Viljoen et al., 2002). Finally, fatness and conformation scores of the carcasses are often reported to increase with an increase in slaughter weight and age at slaughter

(O'Riordan et al., 2011). However, this was not validated in the present study when animals are categorized according to pHu values.

3.1.2. Beef qualities

Since the extreme values were removed, muscle from each pHu cluster would therefore not be expected to be dark in color. The similar muscle L^* between the clusters supports this observation. On another hand, neither tenderness nor juiciness scores were significantly different when animals were categorized according to their final muscle pH. These findings are inconsistent with previous research (Jeremiah et al., 1991; Wu et al., 2014), although a tendency (P = 0.08) was found for tenderness with +0.24 score units in pH_u+ cluster. The similar tenderness scores between the pHu clusters may be ascribed to the similar ageing period (14 days) applied for the ribeye steaks, mainly when it concerns LT muscle of young bulls. Accordingly, recent studies reported that regardless of pHu group (low, medium and high), shear force decreased as meat cuts were aged (Lomiwes et al., 2014; Wu et al., 2014). Thus, one can suppose that despite the high insoluble collagen content found in pH_u+ (**Table** 2) and the narrow pHu values between the clusters, the main drivers of the final tenderness are more complex than expected. Meanwhile, it has been hypothesized that the development of beef tenderness is pHu compartmentalized (Lomiwes et al., 2014; Wu et al., 2014). In this trial, it is important to mention that when animals were categorized based on tenderness, juiciness and beef flavor traits, the expected differences in final pH were found (Tables 3-5, and section 3.2).

In contrast to the textural traits, beef flavor scores were the highest in pH_u+ cluster (+0.67 score units than for pHu=). The differences of flavor scores between pHu clusters would be a consequence of biochemical pathways that involve myriad aroma compounds that are pH-dependent (<u>Flores, 2017</u>). More precisely, the Maillard reactions by their pH-dependency believed to be at the origin for roasted aromas, namely heterocyclic compounds (<u>Flores, 2017</u>).

In the highest pHu-cluster, a^* , b^* and C^* -values were the greatest, which all reflect the redness/vividness of meat. These could be linked to a decrease in the rate of oxygen penetration into the meat which lead to reduced oxygen-dependent enzyme activity and thus affected the oxygenation process (<u>Lawrie and Ledward, 2014</u>). The particularity of pHu+cluster, although the narrow pHu-values, would originate from sophisticated and not yet fully known mechanisms. Furthermore, meat with high pH would be darker in color because there

is less free water to reflect light (<u>Hughes et al., 2014</u>). The effect of pH on meat color was extensively studied. Nevertheless, it is important to consider that controversial findings were reported across studies concerning the linear or curvilinear relationships (<u>Calnan et al., 2016</u>; <u>Gagaoua et al., 2018b</u>).

3.1.3. Animal characteristics and rearing factors

Seven of the 12 animal characteristics and rearing factors were significantly different between pHu clusters (**Table 2**). Among the findings, initial BW and total forage intake were exclusively the highest in pH_u– cluster and were different for the other clusters (pH_u= and pH_u+). Most studies have tested the objective impact of different rearing factors on meat sensory quality, but the impact on the final beef pH has seldom been reported. Although significant, the differences in animal age between the pHu clusters were small (Diff = 1.17 months between pH_u– and pH_u+). Age-related changes in ultimate pH and color traits, have been reported in earlier studies (<u>Furnival et al., 2009</u>). However, because the pHu values varied from 5.48 to 5.70 (0.22 pH units), this should not represent an increased negative impact on meat quality (<u>Bureš and Bartoň, 2012</u>). This was also supported by similar evaluation of meat sensory traits between pHu clusters.

The highest initial BW of the animals of pH_u- cluster would be a consequence of the differences found in feeding by high total forage intake and low percentage of concentrate, respectively, and of slightly high age at slaughter compared to pH_u+. To our knowledge, no study has linked neither initial nor final BW with muscle pHu. However, effects were reported with carcass characteristics (Oury et al., 2007; Soulat et al., 2016) and meat quality traits (Keane and Allen, 1998). We expect that the same reasons discussed above for carcass and muscle carcass weights were behind this finding. Energy intake was described to affect growth rates, body and carcass composition (Hoch and Agabriel, 2004). In line to these and to our hypothesis, the numerically greater energy intake found in pH_u+ and pH_u= clusters compared to pH_u- may have stemmed from cumulative factors over the long fattening period (approximately 200 vs. 176 days). Thus, it seems that short fattening duration of the old animals (Table 2) are likely to give low pHu that can be attributed to the high glycolytic potential of muscle (high LDH activity). In young bulls, the increase with age of the relative glycolytic energy metabolism in muscle to the detriment of the percentage of oxidative fibers has been previously reported (Schreurs et al., 2008). In addition, the low oxidative properties would be related to the low percentage of concentrate observed in pH_u- cluster compared to

pHu+. For example, animals fed with higher concentrate proportions present lower marbling and thus, lower oxidative properties compared with animals receiving high levels of forage (Cooke et al., 2004). The inverse was reported in the case of young bulls as the earlier studies reported lesser glycogen, higher pH, and darker color when the animals were fed a forage-limited diet compared to those fed concentrates *ad libitum* (Vestergaard et al., 2000). Thus, the increase in muscle oxidative metabolism (by ICDH activity in this trial) possibly allowed the decreased necessity to store comparable amounts of muscle glycogen as for a muscle with a higher glycolytic capacity.

3.2. Tenderness, juiciness and beef flavor clusters

The clustering methods allowed the identification of 3 sensory eating quality clusters for tenderness (**Table 3**), juiciness (**Table 4**) and beef flavor (**Table 5**). Following the same procedure than for pHu, the silhouette widths were used for the validation of both the clustering methods and the number of clusters for each sensory trait. For tenderness ($S_i = 0.47$ by HCA vs. 0.51 by k-means) and juiciness ($S_i = 0.54$ by HCA vs. 0.66 by k-means), the retained clusters were those obtained by k-means algorithm and for beef flavor ($S_i = 0.61$ by HCA vs. 0.49 by k-means), they were those of HCA. For the 3 sensory traits, several similarities and tendencies of the direction were detected among the variables that differ between low (–), medium (=) and high (+) clusters. Thus, we have grouped the three traits together when possible and discussed in the following sections the similarities and the few specific differences found between the 3 sensory trait clusters.

For tenderness clusters (**Table 3**), the tough cluster [TEND–, grouped 108 animals (35.06%)] had an average value of 4.14 (± 0.57). The medium tender cluster [TEND=, grouped 141 animals (45.78%)] had an average value of 5.46 (± 0.43). The tender cluster [TEND+, grouped 59 animals (19.16%)] had an average value of 6.95 (± 0.62).

For juiciness clusters (**Table 4**), the dry cluster [JUIC–, grouped 127 animals (41.23%)] had an average value of 4.30 (± 0.37). The medium juicy cluster [JUIC=, grouped 97 animals (31.49%)] had an average value of 5.31 (± 0.33). The juicier cluster [JUIC+, grouped 84 animals (27.27%)] had an average value of 6.60 (± 0.53).

For beef flavor clusters (**Table 5**), the less flavorful cluster [FLAV–, grouped 139 animals (45.13%)] had an average value of 4.12 (± 0.45). The medium flavorful cluster [FLAV=, grouped 71 animals (23.05%)] had an average value of 5.38 (± 0.27). The flavorful cluster [FLAV+, grouped 98 animals (31.82%)] had an average value of 6.40 (± 0.37).

The details of differences among the defined clusters (tenderness, juiciness and flavor) for the continuum data are depicted in **Tables 3** – **5** and summarized for quick interpretation of the data in **Fig. 1**. In the following sections, the consistencies (similarities) and the specificities among the three quality traits clusters are presented and discussed.

3.2.1. Similar and specific differences in carcass characteristics within tenderness, juiciness and beef flavor trait clusters

The hypothesis that among carcass characteristics some of them would have an impact on the palatability of the beef cuts was very strongly supported by our findings. As expected, % fat carcass, fat carcass weight of the 6th rib and fatness carcass score were significantly the greatest (P < 0.01) in tender, juicy and flavorful meat clusters. The 3 variables are different estimates of fat carcass content and lead to the same categorization irrespective of the sensory trait. For the 3 sensory clusters, carcasses with fatness scores greater than 3.0 lead to more tender, juicier and flavorful meat. This confirms the proposal to use the subjective grading of carcasses by their EUROP fat cover scores as a threshold in both the genetic evaluations and carcasses segregation (Varona et al., 2009). These findings evidence also the power of fatness scoring to categorize beef sensory traits contrary to what has been recently reported (Bonny et al., 2016). In the current study, muscle IMF contents agreed to the above results and were respectively 1.56, 1.57 and 2.62-folds higher in tender, juicy and flavorful meat clusters than the tough, dry and less flavorful meat clusters (Tables 3 - 5). These findings agree with the important role of fat in the palatability of beef (Savell and Cross, 1988). The earlier studies proposed that, in order to obtain beef with acceptable palatability, a 3% IMF content (on fresh muscle) is needed; lower amounts would result in tougher, drier and less flavorful beef cuts (Savell and Cross, 1988). This would suggest that IMF affects directly juiciness and flavor attributes; otherwise, tenderness is influenced indirectly by the disorganization or dilution of the structure of collagen content that forms the basal toughness (Nishimura, 2015). Fat would be also involved in meat texture (tenderness and juiciness) through the lubrication theory (Savell and Cross, 1988), which states that fat or marbling present in and around the muscle fibers and perimysium during mastication results in a more tender and juicier beef (Savell and Cross, 1988; Hocquette et al., 2012; Gagaoua et al., 2016b).

Inversely to fat estimates, the %muscle carcass was the highest in FLAV- cluster compared to FLAV+. For tenderness and juiciness clusters, differences were observed but with confounded effect with the medium cluster. This might highlights trait dependency of the

relationships within the significant different variables from the continuum data as the responses to the effects are not always in the same rate and direction (**Tables 3 – 5** and **Fig.1**). The slight increase in the %muscle carcass in FLAV– cluster would be attributed to the decreased fat deposition following at least two principles. First, earlier studies reported that in highly marbled muscle, fat cells replace muscle fibers and reduce protein content in muscle (Albrecht et al., 2011). Accordingly, cell culture conducted by our group showed that adipocyte and muscle fiber developments are reciprocally regulated and influence each other (Bonnet et al., 2010). Second, greater lean meat content and lower fat content in bull carcasses would be assigned to the levels of testosterone, which stimulates amino acid incorporation into proteins, thus increasing muscle weight rather than fat deposition (Dayton and White, 2008).

The %bone carcass, dressing% and LT muscle weight were unable to categorize animals into clusters regardless of the sensory trait. Moreover, the conformation scores were equal between the clusters and did not differ among the sensory traits studied. For instance, these agree to the findings by (Bonny et al., 2016) who also found no relationships between European conformation scores and beef tenderness. Generally, an increase in muscle weight is linked to an increase in the cross-sectional area of the individual muscle fibers with advancing age (Jurie et al., 2005). However, we failed to find any difference in this study when animals are categorized by their final sensory eating qualities or when age was used to cluster the animals into groups (data not shown).

3.2.2. Similar and specific differences in other beef qualities within tenderness, juiciness and beef flavor trait clusters

pHu was the highest in TEND+, JUIC+ and FLAV+ and allowed an efficient discrimination (P < 0.01) between the clusters. If only extreme groups were compared, L^* also discriminated between high (+) and low (-) clusters irrespective of the sensory quality but with strong effect for TEND+ and TEND- (**Table 3**). For both parameters, the average values for pHu were 5.62 ± 0.01) and 5.55 ± 0.01 , and for those of L^* were 37.95 ± 0.01) and 34.15 ± 0.21 , respectively for the high (+) and low (-) sensory trait clusters. The ability of pHu in this study to significantly categorize the extreme clusters (**Fig. 1**) of all the assessed quality traits might partly be attributed to protein denaturation. Previous studies demonstrated that the extent of pH decline plays a role in the spacing of the lattice and myofibril of the muscle (structural changes) and was proposed as a causative driver of light scattering, hence

of final tenderness (Hughes et al., 2014). It was further linked to the reaction of oxygen with myoglobin during meat blooming, thus of color (Wyrwisz et al., 2016). These are mainly supported by the ageing period where muscles tend to become lighter in color due to proteolysis (Kołczak et al., 2003). However, still little is known about the mechanisms behind the effects of ageing on beef color, flavor and juiciness development compared to tenderness attribute. Meanwhile, some studies report more tender meat when pHu is high (Silva et al., 1999), whereas others report more tender meat when the carcasses have low pHu values (Wulf et al., 2002). Although others found that, less tender meat was within intermediate pHu values (Watanabe et al., 1996). An earlier study using a total of 3435 beef carcasses including bulls, heifers and steers, showed the usefulness of both muscle color and pHu for segregating carcasses into tenderness clusters (Jeremiah et al., 1991). In this study and among the color traits, only L^* was able to categorize young bulls cuts into extreme tender groups (tender vs. tough) and there was no relevant significant differences for b^* and hue angle (h^*) within the 3 sensory clusters and for a^* and C^* within both tenderness and juiciness clusters. These findings agree to previous studies, as from the colorimetric results, the main changes in the internal surface of the meat occur mostly in L^* , rather than a^* and b^* (Pakula and Stamminger, 2012).

3.2.3. Similar and specific differences in muscle characteristics within tenderness, juiciness and beef flavor trait clusters

IMF contents (discussed above), total collagen and the mitochondrial ICDH activity, are the three variables that discriminated efficiently between the 3 sensory clusters with a slight categorization effect for juiciness. As expected and similarly to IMF content, ICDH was the highest in more tender, juicier and more flavorful beef clusters, whilst total collagen was the lowest. These findings are in accordance to those reported by earlier studies stating that oxidative properties of muscle lead generally to tender, juicy and flavorful meat (<u>Dransfield et al., 2003</u>; <u>Picard et al., 2014</u>). Nevertheless, it is worth mentioning that controversies exist between studies and the results are likely to be muscle, breed and animal type dependent (<u>Picard et al., 2014</u>; <u>Gagaoua et al., 2016</u>; <u>Gagaoua et al., 2017b</u>). Moreover, the associations are linear or curvilinear dependent (<u>Calnan et al., 2016</u>; <u>Gagaoua et al., 2018b</u>).

Neither glycolytic (LDH) nor total fiber area were able to differentiate the meat quality clusters. Similar findings were reported by (<u>Vestergaard et al., 2000</u>) and (<u>Dransfield et al., 2003</u>) with the glycolytic potential of LT muscle. From this point of view and as discussed

above, pH measurements alone can be used to follow glycolytic changes in muscles. The results concerning total collagen, the major component of muscle connective tissue, has been the target of numerous studies in relation with meat tenderness and our findings are in line to the negative link with tenderness (<u>Dransfield et al., 2003</u>; <u>Jurie et al., 2005</u>; <u>Dubost et al., 2013</u>; <u>Nishimura, 2015</u>; <u>Gagaoua et al., 2016</u>; <u>Listrat et al., 2016</u>). Several of these studies attributed decrease in tenderness to the increase in collagen content with increasing age at slaughter. This hypothesis was strongly confirmed by our data as the low tenderness clusters had the greatest total collagen content and the carcasses were the heaviest with high muscle and low fat contents. From the other muscle characteristics, insoluble collagen content distinguished slightly the flavorful cluster only from both others but made no distinction between low and medium flavor clusters.

3.2.4. Similar and specific differences in animal characteristics and rearing factors within tenderness, juiciness and beef flavor trait clusters

The most influenced rearing factors regardless the sensory trait were initial BW, fattening duration, forage% and DMI. The two former factors were further influenced by pHu clustering (Fig. 1) as discussed above. The fattening period refer to the growing of animals to a desired end-point, that is mainly defined on the basis of a combination of live-weight, carcass weight and degree of marbling of the beef (Muir et al., 1998). For the three sensory traits, approximately a 12% lower initial BW between the high (+) and low (-) quality clusters (average weight for the high quality clusters is \leq 372 kg), gave more tender, juicy and flavorful meat. This is an interesting finding, as it would be easily used for joint rearing practices of the fattening period to enhance the sensory qualities of meat cuts during a low fattening period, approximately ~156 days for the high (+) vs. ~179 days for the low (-) quality clusters. In agreement, the effect is more pronounced within the animals fed with high concentrate, as found in this study for TEND+ and FLAV+ clusters (Table 3 and 5). However, it seems that the decrease of the fattening duration had little detrimental effect on carcass characteristics. This would be true as the low quality clusters grouped mainly all the animals with the better carcass characteristics (the highest muscle carcass%, carcass weight, muscle carcass weight in kg and final BW), but without any difference on carcass conformation score irrespective of the sensory trait cluster. Using a predictive approach, a recent work by our group showed that the fattening period duration and initial BW were also important rearing factors in the fat tissue and muscle percentages of young bulls (Soulat et al., 2016).

Notable among the findings for the high clusters grouping mainly the youngest animals is the fatness scores, which were the greatest (>3.0) as discussed above and lead in all cases to tender, juicy and more flavorful beef. We suppose that the great effect would be confounded within the age at slaughter of the animals. In this context, it was proposed to analyze the age and weight at slaughter together because, a greater weight implies a higher age as it is the case in this study, except when feed or fattening conditions are manipulated (Jurie et al., 2005). Accordingly, slaughtered at different ages, the meat of the youngest bulls (~16 months) with the lighter carcasses were clustered in the TEND+ cluster and was clustered as juicier and flavorful compared to the meat cuts of TEND= and TEND— clusters. In an earlier comprehensive review and among different studies, it was concluded that acceptable carcass weights and degrees of maturity could be achieved at young ages (Muir et al., 1998). This was validated in our case with original information on characteristics referring to the farm-to-table continuum.

For the two other discriminant variables of eating quality clusters, the more tender, juicier and more flavorful meats were obtained with animals that had the lowest DMI (<8.22 kg DM/day). DMI is linearly related with the composition of diet (Juniper et al., 2007) and was among the rearing factors that were retained in the equations models of fat tissue and muscle percentage prediction for both cows and young bulls (Soulat et al., 2016). In the present study, the link of DMI with meat sensory qualities in addition to the link with carcass characteristics were confirmed. This agrees with the reduction of DMI when the proportion of concentrates in the diet was increased (Steen and Kilpatrick, 2000). This was recently validated in the prediction models developed by our group (Soulat et al., 2016). On another hand, forages provide energy and the essential nutrients at approximately half the cost of grain-based feedlot rations (Muir et al., 1998). Thus, fattening young bulls and even to a fattening period of 156 days and initial BW that not exceed 372 kg, by allocating them at both forages and concentrates would allow an option for increased productivity, profit to producers and satisfaction of consumers with the greatest sensory qualities as depicted in this trial. Moreover, in this study, the 54 kg greater final BW for the TEND- cluster translated into 32 kg greater carcass weight, was not associated with improved carcass conformation. Increased carcass weight has both been associated (Keane et al., 2006) and not associated (Keane and Allen, 2002) with improved carcass conformation or sensory quality traits (Steen and Kilpatrick, 2000; Jurie et al., 2005). Inversely to our findings, previous studies on bulls and/or steers showed that longer fattening durations improve carcass conformation and

fatness scores (Keane and Allen, 1998). From the other rearing factors, neither body weight gain nor feed efficiency were different and able to differentiate between meat sensory qualities. This is in contrast to the findings by (Purchas et al., 2002) who found that an increase in daily weight gain of young bulls and steers lead to a significant increase in meat tenderness. The same was found with energy intake, which was found to be different and the highest in FLAV+ cluster only. In literature, many reports have emphasized a decrease in shear force when the feed ration energy level increases (Keane and Allen, 1998; Purchas et al., 2002; Juniper et al., 2007). Overall, from these findings it seems that data related to total animals' live, namely those of the fattening period, are of interest to categorize animals into sensory quality clusters. They would be further used for carcasses potential estimation. The management of factors likely related to the fattening period would be helpful to enhance the eating quality potential of animals.

4. Conclusions

This study showed that implementation of a holistic approach by considering animal characteristics and the farm-to-table data, named as the continuum, is a promising approach since it allows characterizing the multifactorial effects that need joint management to achieve the desirable beef eating qualities. The use of animal characteristics and rearing factors together with carcass and muscle characteristics allowed to identify different variables as potential discriminators of high and low beef sensory qualities. This is of great importance because they can be proposed to the wholesalers and retailers of beef to achieve the quality expectations if combined with accurate statistical analyses such as decision trees (Gagaoua et al., 2018a) and prediction equations (Soulat et al., 2016; Gagaoua et al., 2017b; Gagaoua et al., 2018b). Overall and from the findings of this study, the categorization of the meat cuts by their pHu showed divergences in carcass properties and muscle carcass weights, however, neither tenderness nor juiciness scores were different. Accordingly, amongst the animal and rearing factors, low pH_u (-) cluster grouped the old animals that were fattened at high initial BW and forage intake. On the contrary, fattening duration and energy intake were the lowest for this cluster compared to the medium and high pH clusters. For muscle characteristics, lactate dehydrogenase was the only variable that efficiently discriminated pHu clusters with high activity for pH_u - cluster compared to the two others. For the palatability meat traits, *i.e.*, tenderness, juiciness and flavor, different variables from the continuum data were found to discriminate or to be different among the quality clusters. The most discriminative were fat estimates. The findings of this trial evidenced the power of fatness scoring to categorize beef

sensory traits. Irrespective of the sensory clusters, carcasses with fatness scores greater than 3.0 lead to more tender, juicier and more flavorful meat. From the rearing factors, the best and interesting discriminators were fattening duration and DMI. These suggest that a joint management at the farm level to achieve the desirable qualities would be possible. Finally, this trial highlighted the different effects at each level of the farm-to-table continuum (animal and rearing factors, carcass, muscle and meat) on the palatability traits by identifying significant variables that would be used in the future for prediction purposes by beef stakeholders of meat and carcass qualities.

Conflict of interest

The authors declare that no competing interests exist.

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Figure captions

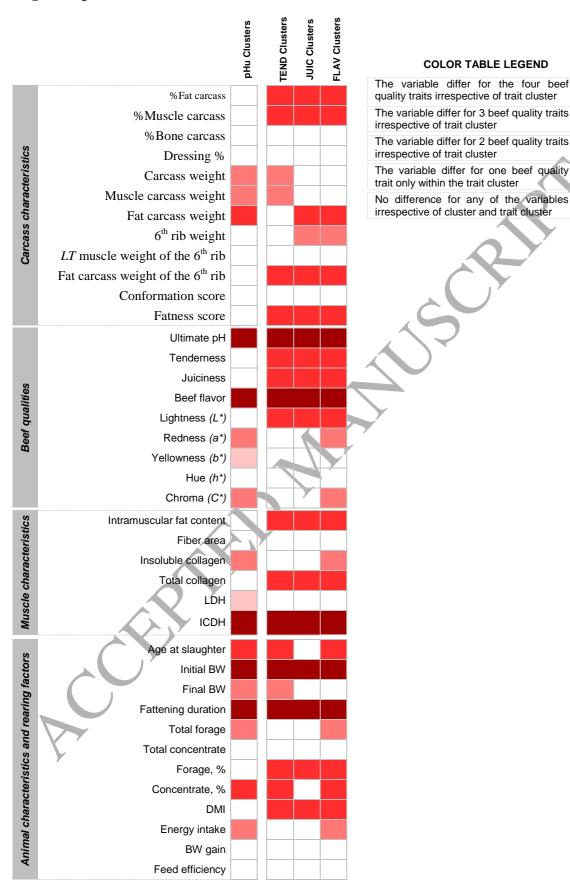


Fig. 1

Fig. 1. A summary of the differential variables from **Tables 2** - **5**, found for each eating quality cluster (pHu, tenderness, juiciness and beef flavor). A red color key (gradient ruler) on the upper left indicates the level of the effect of clustering on the studied variables for each quality cluster trait.

Table 1. Description of the list of the 39 variables corresponding to carcass characteristics, meat eating quality traits, muscle characteristics, animal characteristics and rearing factors data measured for the studied French young bulls.

	n	Mean	SD^1	Min	Max
Carcass characteristics					
%Fat carcass	480	14.97	3.07	7.26	25.86
% Muscle carcass	480	70.79	3.43	60.83	79.50
%Bone carcass	480	14.25	1.25	11.05	18.78
Dressing, %	480	58.82	3.09	49.50	67.98
Carcass weight, kg	480	393.7	64.8	260.5	652.2
Muscle carcass weight, kg ²	480	285.4	50.3	170.3	460.3
Fat carcass weight, kg ²	480	60.67	19.09	27.44	157.4
6 th rib weight, kg	480	2.37	0.48	1.46	4.45
LT muscle weight of the 6 th rib, kg	480	0.31	0.07	0.12	0.59
Fat carcass weight of the 6 th rib, kg	480	0.32	0.03	0.07	0.88
Conformation score, $1 - 15$ scale ³	437	10.45	1.11	7.00	13.00
Fatness score, 1 – 5 scale ⁴	437	2.56	0.56	1.66	4.00
Beef qualities					
Ultimate pH	377	5.59	0.14	5.10	6.36
Tenderness, 0 –10 scale ⁵	308	5.5	1.2	1.8	9.2
Juiciness, $0 - 10$ scale	308	5.2	1.0	2.6	8.1
Beef flavor, $0 - 10$ scale	308	5.1	1.1	2.9	7.4
Lightness $(L^*)^6$	290	36.59	4.19	25.61	47.64
Redness $(a^*)^7$	290	13.82	2.44	7.79	23.07
Yellowness $(b^*)^8$	290	15.22	3.33	8.98	26.19
Hue $(h^*)^9$	290	47.46	5.45	33.58	58.52
Chroma (<i>C</i> *) ¹⁰	290	20.64	3.68	14.11	34.90
Muscle characteristics					
Intramuscular fat content, (g 100 g ⁻¹ wet tissue)	321	1.82	1.02	0.78	5.06
Fiber area, μm ²	310	3228	642	1729	5623
Insoluble collagen, μg OH-prol mg ⁻¹ DM	312	3.18	0.81	1.39	5.74
Total collagen, µg OH-prol mg 1 DM	418	3.19	0.73	1.60	6.21
Lactate dehydrogenase (LDH), μmol min ⁻¹ g ⁻¹	427	958	165	508	1475
Isocitrate dehydrogenase (ICDH), µmol min ⁻¹ g ⁻¹	427	1.38	0.55	0.40	3.10
Animal characteristics and rearing factors					
Age at slaughter, month	480	16.66	2.30	13.01	25.59
Initial BW, kg	480	383.3	53.9	266.0	567.3
Final BW, kg	480	669.0	103.0	479.0	1066.5
Fattening duration, days	480	188.8	62.4	105.0	328.0
Total forage intake, kg DM/day	268	3.90	2.59	0.45	9.58
Total concentrate intake, kg DM/day	268	4.51	2.41	1.17	8.85
Forage, %	480	60.86	19.58	6.51	85.79
Concentrate, %	480	39.14	27.03	14.21	93.49
Dry matter intake (DMI), kg DM/day	480	9.53	2.00	5.53	14.59
Energy intake, Mcal/day	480	15.10	2.73	7.48	20.66
BW gain (average daily gain-ADG), kg/d	326	1.28	0.29	0.52	2.12
Feed efficiency (ADG:DMI ratio), kg/kg DM	326	144.6	32.9	56.9	222.3
1 ~ ~					

¹ SD: Standard deviation

 $^{^2}$ Muscle and fat carcass weights were estimated from the 6^{th} rib composition obtained by dissection as in (<u>Gagaoua et al., 2018b</u>). Fat weight is the sum of internal, subcutaneous and intermuscular fat

weights.

$$^{10}C^* = [(a^{*2} + b^{*2})^{1/2}].$$

 $^{^{3}}$ EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

⁴ EUROP classification grid for carcass fatness scores from 1= leanest to 5 = fattest.

⁵ Score of 0 extremely (tough, dry, and weak) to a score of 10 extremely (tender, juicy and strong).

 $^{^6}L^*$ is the lightness on a scale of 0 (black) to 100 (white).

 $^{^{7}}a*$ spans 197 from +60 (red) to -60 (green).

 $^{^8}b*$ spans from +60 (yellow) to -60 (blue).

 $^{^{9}}h^{*} = [(b^{*}/a^{*}) \tan^{-1}].$

Table 2. Comparison of carcass characteristics, meat eating quality traits, muscle characteristics and animal characteristics and rearing factors, of the three ultimate pH clusters identified by the hierarchical clustering using Ward's minimum variance method.

	pH _u –	pH _u =	pH _u +		
$n = \frac{1}{n}$	145	144	79	SEM	<i>P</i> -value ¹
Ultimate pH mean ± SD	5.48 ± 0.06	5.59 ± 0.03	5.70 ± 0.08	0.006	***
Carcass characteristics					
%Fat carcass	15.12	14.93	14.30	0.17	ns
%Muscle carcass	70.97	71.20	71.77	0.17	ns
%Bone carcass	13.91	13.89	13.96	0.05	ns
Dressing, %	58.96	59.07	59.35	0.17	ns
Carcass weight, kg	412.54 ^a	410.63 ^a	387.24 ^b	3.33	*
Muscle carcass weight, kg ²	299.90 ^a	298.70 ^a	284.51 ^b	2.49	*
Fat carcass weight, kg ²	64.56	63.55	66.62	1.07	ns
6 th rib weight, kg	2.46	2.42	2.41	0.02	ns
LT muscle weight of the 6 th rib, kg	0.33	0.32	0.33	0.003	ns
Fat carcass weight of the 6 th rib, kg	0.34	0.33	0.30	0.007	ns
Conformation score, 1 – 15 scale ³	10.46	10.55	10.60	0.05	ns
Fatness score, 1 – 5 scale ⁴	2.48	2.57	2.61	0.03	ns
Beef qualities	Á				
Tenderness, 0 – 10 scale ⁵	5.3 ^b	5.3 ^b	5.5 ^a	0.07	t
Juiciness, 0 – 10 scale	5.3	5.1	5.4	0.07	ns
Beef flavor, 0 − 10 scale	5.1 ^b	4.8°	5.4ª	0.07	**
Lightness $(L^*)^6$	37.22	36.82	35.83	0.26	ns
Redness $(a^*)^{7}$	13.84 ^b	13.50 ^b	14.41 ^a	0.15	*
Yellowness (b*) 8	14.98 ^b	14.77 ^b	16.23 ^a	0.21	*
Hue $(h^*)^9$	47.01	47.22	48.17	0.34	ns
Chroma (C*) 10	20.48^{b}	20.11 ^b	21.78^{a}	0.23	*
Muscle characteristics					
Intramuscular fat content, (g 100 g ⁻¹ wet tissue)	1.79	1.80	1.85	0.09	ns
Fiber area, μm^2	3215	3351	3431	42.4	ns
Insoluble collagen, μg OH-prol mg ⁻¹ DM	2.93°	3.23 ^b	3.51 ^a	0.05	***
Total collagen, μg OH-prol mg ⁻¹ DM	3.39	3.22	3.21	0.04	ns
Lactate dehydrogenase (LDH), µmol min ⁻¹ g ⁻¹	959.2 ^a	930.1 ^b	909.3°	7.67	*
Isocitrate dehydrogenase (ICDH), μmol min ⁻¹ g ⁻¹	1.20 ^b	1.26 ^b	1.42 ^a	0.03	**
Animal characteristics and rearing factors					
Age at slaughter, month	17.09 ^a	16.90 ^a	15.92 ^b	0.13	**
Initial BW, kg	397.91 ^a	388.25 ^b	362.35°	2.99	***
Final BW, kg	698.80^{a}	695.84 ^a	653.46 ^b	5.34	**
Fattening duration, days	176.62 ^b	198.81 ^a	201.46 ^a	3.44	***
Total forage intake, kg DM/day	4.34 ^a	$4.05^{\rm b}$	2.54 ^c	0.20	**
Total concentrate intake, kg DM/day	4.78	4.75	5.30	0.18	ns
Forage, %	62.01	63.89	54.74	1.25	ns
Concentrate, %	37.47 ^b	36.21 ^b	46.26 ^a	1.45	*
Dry matter intake (DMI), kg DM/day	9.71	10.14	9.58	0.11	ns
Energy intake, Mcal/day	14.99 ^b	15.81 ^a	15.82 ^a	0.13	*
BW gain (average daily gain-ADG), kg/d	1.29	1.39	1.33	0.03	ns
Feed efficiency (ADG:DMI ratio), kg/kg DM	139.81	145.20	142.78	3.57	ns

a.b,c Least-square means in the same row with different superscript letters are significantly different (P < 0.05).

¹ Significances: ns: not significant; tendency (t), P = 0.08; * P < 0.05; ** P < 0.01; *** P < 0.001

$$^{10}C^* = [(a^{*2} + b^{*2})^{1/2}].$$

 $^{^2}$ Muscle and fat carcass weights were estimated from the 6^{th} rib composition obtained by dissection as in (<u>Gagaoua et al., 2018b</u>). Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

 $^{^{3}}$ EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

⁴ EUROP classification grid for carcass fatness scores from 1= leanest to 5 = fattest.

⁵ Score of 0 extremely (tough, dry, and weak) to a score of 10 extremely (tender, juicy and strong) for tenderness, juiciness and beef flavors attributes assessed by a trained sensory panel.

 $^{^{6}}L^{*}$ is the lightness on a scale of 0 (black) to 100 (white).

 $^{^{7}}a*$ is the redness that spans from +60 (red) to -60 (green).

 $^{^8}b*$ is the yellowness that spans from +60 (yellow) to -60 (blue).

 $^{^{9}}h* = [(b*/a*) \tan^{-1}].$

Table 3. Comparison of carcass characteristics, meat eating quality traits, muscle characteristics and animal characteristics and rearing factors, of the three tenderness clusters identified by the k-means algorithm.

	TEND-	TEND=	TEND+	~~~	- 1
$n = \frac{1}{n}$	108	141	59	SEM	P-value ¹
Tenderness mean ± SD	4.1 ± 0.6	5.5 ± 0.4	6.9 ± 0.6	0.07	***
Carcass characteristics					
%Fat carcass	14.39 ^c	15.87 ^b	16.47 ^a	0.18	***
%Muscle carcass	71.58 ^a	$69.72^{a,b}$	69.05 ^b	0.21	***
%Bone carcass	14.07	14.44	14.46	0.08	ns
Dressing, %	59.54	59.34	59.48	0.17	ns
Carcass weight, kg	401.63 ^a	392.03 ^{a,b}	369.59 ^b	3.97	*
Muscle carcass weight, kg ²	292.99 ^a	280.08^{b}	262.16 ^c	3.04	**
Fat carcass weight, kg ²	59.16	64.44	63.43	1.21	ns
6 th rib weight, kg	2.39	2.42	2.43	0.03	ns
LT muscle weight of the 6 th rib, kg	0.31	0.32	0.31	0.004	ns
Fat carcass weight of the 6 th rib, kg	0.30^{c}	0.36^{b}	0.38^{a}	0.008	**
Conformation score, $1 - 15$ scale ³	10.44	10.19	10.05	0.07	ns
Fatness score, 1 – 5 scale ⁴	2.42^{c}	2.75^{b}	3.09^{a}	0.04	***
Beef qualities		()			
Ultimate pH	5.44 ^c	5.58 ^b	5.63 ^a	0.01	**
Juiciness, 0 – 10 scale ⁵	4.6°	5.3 ^b	6.3 ^a	0.06	***
Beef flavor, 0 – 10 scale	4.4°	5.3 ^b	6.2^{a}	0.06	***
Lightness $(L^*)^6$	34.21°	36.35 ^b	38.29^{a}	0.43	**
Redness $(a^*)^{7}$	13.87	13.61	13.45	0.25	ns
Yellowness (b*) 8	16.09	15.60	15.64	0.28	ns
Hue $(h^*)^9$	49.17	48.86	49.27	0.47	ns
Chroma (C*) 10	21.30	20.80	20.70	0.34	ns
Muscle characteristics					
Intramuscular fat content, (g 100 g ⁻¹ wet tissue)	1.24 ^c	$1.70^{\rm b}$	1.97^{a}	0.11	**
Fiber area, μm^2	3182	3220	3041	52.3	ns
Insoluble collagen, µg OH-prol mg ⁻¹ DM	2.64	2.64	2.60	0.04	ns
Total collagen, μg OH-prol mg ⁻¹ DM	3.51 ^a	3.20^{b}	3.11 ^c	0.05	**
Lactate dehydrogenase (LDH), µmol min ⁻¹ g ⁻¹	954.0	993.2	960.5	10.7	ns
Isocitrate dehydrogenase (ICDH), μmol min ⁻¹ g ⁻¹	1.31 ^b	$1.50^{a,b}$	1.55 ^a	0.04	*
Animal characteristics and rearing factors					
Age at slaughter, month	17.05 ^a	16.83 ^a	15.99 ^b	0.14	*
Initial BW, kg	411.54 ^a	394.70^{b}	367.17°	3.24	***
Final BW, kg	674.77 ^a	659.71 ^b	620.94°	6.23	**
Fattening duration, days	182.33 ^a	159.38 ^b	155.29 ^b	3.11	**
Total forage intake, kg DM/day	3.94	4.24	3.31	0.17	ns
Total concentrate intake, kg DM/day	4.96	4.30	4.64	0.16	ns
Forage, %	58.11 ^a	53.36 ^a	41.23 ^b	1.50	**
Concentrate, %	41.87 ^b	46.64 ^b	58.77 ^a	1.68	*
Dry matter intake (DMI), kg DM/day	8.99 ^a	8.58 ^b	8.04 ^c	0.07	***
Energy intake, Mcal/day	14.00	14.62	14.28	0.16	ns
BW gain (average daily gain-ADG), kg/d	1.33	1.26	1.24	0.02	ns
Feed efficiency (ADG:DMI ratio), kg/kg DM	144.61	143.56	147.18	2.66	ns

a.b,c Least-square means in the same row with different superscript letters are significantly different (P < 0.05).

¹ Significances: ns: not significant; * P < 0.05; ** P < 0.01; *** P < 0.001: ns: not significant; * P < 0.05; ** P < 0.05; *** P < 0.05; ***

< 0.01; *** *P* < 0.001

$$^{10}C^* = [(a^{*2} + b^{*2})^{1/2}].$$

 $^{^2}$ Muscle and fat carcass weights were estimated from the 6^{th} rib composition obtained by dissection as in (<u>Gagaoua et al., 2018b</u>). Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

 $^{^{3}}$ EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

⁴ EUROP classification grid for carcass fatness scores from 1= leanest to 5 = fattest.

⁵ Score of 0 extremely (tough, dry, and weak) to a score of 10 extremely (tender, juicy and strong) for tenderness, juiciness and beef flavors attributes assessed by a trained sensory panel.

 $^{^{6}}L^{*}$ is the lightness on a scale of 0 (black) to 100 (white).

 $^{^{7}}a*$ is the redness that spans from +60 (red) to -60 (green).

 $^{^8}b*$ is the yellowness that spans from +60 (yellow) to -60 (blue).

 $^{^{9}}h* = [(b*/a*) \tan^{-1}].$

Table 4. Comparison of carcass characteristics, meat eating quality traits, muscle characteristics and animal characteristics and rearing factors, of the three juiciness clusters identified by the k-means algorithm.

	JUIC-	JUIC=	JUIC+		1
$n = \frac{1}{n}$	127	97	84	SEM	<i>P</i> -value ¹
Juiciness mean ± SD	4.3 ± 0.4	5.3 ± 0.3	6.6 ± 0.5	0.06	***
Carcass characteristics					
%Fat carcass	14.68 ^c	15.54 ^b	16.58 ^a	0.18	***
%Muscle carcass	71.02^{a}	70.11 ^a	69.21 ^b	0.21	**
%Bone carcass	14.38	14.28	14.25	0.08	ns
Dressing, %	59.25	59.38	59.78	0.17	ns
Carcass weight, kg	389.18	390.72	394.45	3.97	ns
Muscle carcass weight, kg ²	282.16	280.36	280.63	3.04	ns
Fat carcass weight, kg ²	58.19 ^c	62.66 ^b	68.46 ^a	1.21	**
6 th rib weight, kg	2.31^{b}	2.42^{b}	2.54 ^a	0.03	*
LT muscle weight of the 6 th rib, kg	0.31	0.32	0.32	0.004	ns
Fat carcass weight of the 6 th rib, kg	0.30^{c}	0.35^{b}	0.40^{a}	0.008	***
Conformation score, 1 – 15 scale ³	10.37	10.21	10.13	0.07	ns
Fatness score, 1 − 5 scale ⁴	2.41°	2.77 ^b	3.06^{a}	0.04	***
Beef qualities					
Ultimate pH	5.55 ^b	5.57 ^b	5.61 ^a	0.01	*
Tenderness, 0 – 10 scale ⁵	$4.8^{\rm c}$	5.6 ^b	6.3 ^a	0.07	***
Beef flavor, 0 – 10 scale	4.4 ^c	5.3 ^b	6.1 ^a	0.06	***
Lightness $(L^*)^6$	34.33 ^b	37.11 ^a	37.13 ^a	0.43	**
Redness $(a^*)^7$	12.83	14.40	14.10	0.25	ns
Yellowness $(b^*)^8$	15.55	16.04	15.84	0.28	ns
Hue $(h^*)^9$	50.28	47.94	48.46	0.47	ns
Chroma $(C^*)^{10}$	20.21	21.63	21.30	0.34	ns
Muscle characteristics					
Intramuscular fat content, (g 100 g ⁻¹ wet tissue)	1.30^{c}	1.57 ^b	2.04^{a}	0.08	**
Fiber area, μm^2	3228	3024	32560	52.3	ns
Insoluble collagen, μg OH-prol mg ⁻¹ DM	2.71	2.57	2.59	0.04	ns
Total collagen, μg OH-prol mg ⁻¹ DM	3.45^{a}	3.14^{b}	$3.23^{a,b}$	0.05	*
Lactate dehydrogenase (LDH), µmol min ⁻¹ g ⁻¹	982.3	990.4	939.5	10.7	ns
Isocitrate dehydrogenase (ICDH), µmol min ⁻¹ g ⁻¹	1.38 ^b	1.44 ^b	1.56 ^a	0.04	*
Animal characteristics and rearing factors					
Age at slaughter, month	16.79	16.73	16.71	0.14	ns
Initial BW, kg	404.7^{a}	399.3 ^a	376.6^{b}	3.24	**
Final BW, kg	656.9	659.2	656.7	6.23	ns
Fattening duration, days	178.9^{a}	159.5 ^b	156.4 ^b	3.11	**
Total forage intake, kg DM/day	3.95	3.92	4.01	0.17	ns
Total concentrate intake, kg DM/day	4.77	4.71	4.21	0.16	ns
Forage, %	56.86 ^a	51.25 ^a	48.10^{b}	1.50	*
Concentrate, %	43.14	48.73	51.90	1.68	ns
Dry matter intake (DMI), kg DM/day	8.81^{a}	8.72^{a}	8.22^{b}	0.07	**
Energy intake, Mcal/day	14.16	14.11	14.87	0.16	ns
BW gain (average daily gain-ADG), kg/d	1.29	1.30	1.24	0.02	ns
Feed efficiency (ADG:DMI ratio), kg/kg DM	143.5	144.9	145.9	2.66	ns

a.b,c Least-square means in the same row with different superscript letters are significantly different (P < 0.05).

¹ Significances: ns: not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

$$^{10}C^* = [(a^{*2} + b^{*2})^{1/2}].$$

 $^{^2}$ Muscle and fat carcass weights were estimated from the 6^{th} rib composition obtained by dissection as in (<u>Gagaoua et al., 2018b</u>). Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

 $^{^{3}}$ EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

⁴ EUROP classification grid for carcass fatness scores from 1= leanest to 5 = fattest.

⁵ Score of 0 extremely (tough, dry, and weak) to a score of 10 extremely (tender, juicy and strong) for tenderness, juiciness and beef flavors attributes assessed by a trained sensory panel.

 $^{^{6}}L^{*}$ is the lightness on a scale of 0 (black) to 100 (white).

 $^{^{7}}a*$ is the redness that spans from +60 (red) to -60 (green).

 $^{^8}b*$ is the yellowness that spans from +60 (yellow) to -60 (blue).

 $^{^{9}}h* = [(b*/a*) \tan^{-1}].$

Table 5. Comparison of carcass characteristics, meat eating quality traits, muscle characteristics and animal characteristics and rearing factors, of the three beef flavor clusters identified by the hierarchical clustering using Ward's minimum variance method.

	FLAV-	FLAV=	FLAV+		
$n = \frac{1}{n}$	139	71	n = 98	SEM	<i>P</i> -value ¹
Beef flavor mean ± SD	4.1 ± 0.4	5.4 ± 0.3	6.4 ± 0.4	0.06	***
Carcass characteristics					
	1.4.126	15 50h	1 < < 48	0.10	ale ale ale
%Fat carcass	14.12°	15.50 ^b	16.64 ^a	0.18	***
%Muscle carcass	71.70 ^a	69.00 ^b	69.08 ^b	0.21	***
%Bone carcass	14.23	14.47	14.32	0.08	ns
Dressing, %	59.82	58.52	59.55		ns
Carcass weight, kg	391.68	397.87	385.36	3.97	ns
Muscle carcass weight, kg ²	285.54	283.61	273.22	3.04	ns
Fat carcass weight, kg ²	55.82 ^b	67.33 ^a	68.48 ^a	1.21	***
6 th rib weight, kg	2.29^{b}	2.49^{a}	2.53 ^a	0.03	**
LT muscle weight of the 6 th rib, kg	0.31	0.32	0.33	0.004	ns
Fat carcass weight of the 6 th rib, kg	0.28^{b}	0.39^{a}	0.40^{a}	0.008	***
Conformation score, $1 - 15$ scale ³	10.36	10.07	10.24	0.07	ns
Fatness score, 1 – 5 scale ⁴	2.36^{c}	2.83^{b}	3.09^{a}	0.04	***
Beef qualities		4 / ~			
	5.55 ^b	5.54 ^b	5.63 ^a	0.01	**
Ultimate pH		5.6 ^b	6.4^{a}		***
Tenderness, 0 – 10 scale	4.7°	5.5 ^b		0.07	***
Juiciness, 0 – 10 scale	4.5°		6.1 ^a	0.06	
Lightness $(L^*)^6$	33.92 ^b	37.10 ^a	38.42 ^a	0.43	***
Redness $(a^*)^7$	12.54°	15.25 ^a	14.13 ^b	0.25	**
Yellowness $(b^*)^8$	15.19	16.92	15.80	0.28	ns
Hue $(h^*)^9$	50.02	48.14	48.32	0.47	ns
Chroma $(C^*)^{10}$	19.77 ^c	22.84 ^a	21.27^{b}	0.34	**
Muscle characteristics					
Intramuscular fat content, (g 100 g ⁻¹ wet tissue)	0.88^{c}	2.06^{b}	2.32^{a}	0.09	***
Fiber area, μm ²	3246	3066	3144	52.3	ns
Insoluble collagen, µg OH-prol mg ⁻¹ DM	$2.64^{a,b}$	2.76^{a}	2.53^{b}	0.04	*
Total collagen, μg OH-prol mg ⁻¹ DM	3.43^{a}	3.24 ^b	3.14^{c}	0.05	*
Lactate dehydrogenase (LDH), µmol min ⁻¹ g ⁻¹	974.9	991.0	957.7	10.7	ns
Isocitrate dehydrogenase (ICDH), μmol min ⁻¹ g ⁻¹	1.31 ^c	1.49 ^b	1.69 ^a	0.04	***
Animal characteristics and rearing factors					
Age at slaughter, month	16.77 ^b	17.37 ^a	16.27 ^b	0.14	*
Initial BW, kg	422.72 ^a	373.51 ^b	372.31 ^b	3.24	***
/ ·	654.18	679.50	646.47	6.23	
Final BW, kg	177.62 ^a	167.57 ^b	157.37°	3.11	ns *
Fattening duration, days	3.59^{b}				***
Total forage intake, kg DM/day		5.50 ^a	3.35°	0.17	
Total concentrate intake, kg DM/day	4.25	4.05	4.78	0.16	ns ***
Forage, %	51.95 ^b	70.01 ^a	41.23°	1.50	
Concentrate, %	48.03 ^b	29.99 ^c	58.77 ^a	1.68	***
Dry matter intake (DMI), kg DM/day	8.95 ^a	8.58 ^b	8.19 ^c	0.07	***
Energy intake, Mcal/day	13.75 ^b	14.33 ^{a,b}	14.78 ^a	0.16	*
BW gain (average daily gain-ADG), kg/d	1.33	1.27	1.22	0.02	ns
Feed efficiency (ADG:DMI ratio), kg/kg DM	143.98	149.17	142.24	2.66	ns

a.b,c Least-square means in the same row with different superscript letters are significantly different (P < 0.05).

¹ Significances: ns: not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

$$^{10}C^* = [(a^{*2} + b^{*2})^{1/2}].$$

 $^{^2}$ Muscle and fat carcass weights were estimated from the 6^{th} rib composition obtained by dissection as in (<u>Gagaoua et al., 2018b</u>). Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

 $^{^{3}}$ EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

⁴ EUROP classification grid for carcass fatness scores from 1= leanest to 5 = fattest.

⁵ Score of 0 extremely (tough, dry, and weak) to a score of 10 extremely (tender, juicy and strong) for tenderness, juiciness and beef flavors attributes assessed by a trained sensory panel.

 $^{^{6}}L^{*}$ is the lightness on a scale of 0 (black) to 100 (white).

 $^{^{7}}a*$ is the redness that spans from +60 (red) to -60 (green).

 $^{^8}b*$ is the yellowness that spans from +60 (yellow) to -60 (blue).

 $^{^{9}}h* = [(b*/a*) \tan^{-1}].$

Highlights

- Unsupervised learning tools are very powerful for the categorization of meat into quality clusters.
- The farm-to-table continuum as a promising approach for better understanding of the variability in meat sensory traits.
- Animal characteristics and rearing factors are related with eating qualities of young bulls beef.
- Carcass characteristics, namely fatness score, discriminated between high and low sensory qualities of beef.
- Joint management of desirable potential qualities of beef (meat and carcass) using data from the different levels of the farm-to-table continuum is possible.

