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## **Certain relationships between Animal Performance, Sensory Quality and Nutritional Quality can be generalized between various experiments on animal of similar types**

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### ► To cite this version:

Marie-Pierre Ellies-Oury, Denys Durand, Anne Listrat, Marie Chavent, Jérôme Saracco, et al.. Certain relationships between Animal Performance, Sensory Quality and Nutritional Quality can be generalized between various experiments on animal of similar types. *Livestock Science*, 2021, 250, pp.104554. 10.1016/j.livsci.2021.104554 . hal-03272625

**HAL Id: hal-03272625**

**<https://hal.science/hal-03272625v1>**

Submitted on 13 Jun 2023

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1        **Certain relationships between Animal Performance, Sensory Quality and Nutritional**  
2        **Quality can be generalized between various experiments on animal of similar types**

3  
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14  
15  
16       **Abstract**

17       In the beef sector, one of the major challenges is to early predict carcass and meat quality and to  
18       jointly satisfy the multiple expectations of the various stakeholders. Thus, the objective of this  
19       study was to determine if the relationships among carcass, nutritional and sensory qualities  
20       established previously by Ellies-Oury et al. (2016) might be generalized to different type of  
21       animals.

22       The *Longissimus thoracis* muscles of 32 young Charolais bulls were analyzed in terms of sensory  
23       and nutritional quality (lipid content and fatty acid composition). These parameters of interest  
24       were linked together and to animal performances by using a clustering of variables.

25 *Longissimus thoracis* sensory and nutritional qualities appear sometimes antagonistic. Indeed,  
26 some “positive” sensory descriptors (juiciness, overall appreciation, overall flavor and overall  
27 odor) are negatively related to PUFA proportions. PUFA proportions are positively associated  
28 with carcass weight but in the same time with rancid/fish flavors. Moreover, CLA and *trans*  
29 MUFA proportions are positively associated with the “negative” descriptors of greasy feel and  
30 residues. To finish, carcass weight and ADG are negatively associated with some “positive”  
31 sensory descriptors except tenderness.

32 It can be concluded from this work that these relationships, that were already established in  
33 previous works, are robust between experiments.

34 In order to highlight robust and generalizable relationships in different contexts, it is now  
35 appropriate to apply this method to a larger database containing different traits and various  
36 characteristics of breed, slaughter ages, animal types, fattening practices, ...

37

### 38 **Keywords**

39 clustering of variables, animal performances, meat quality traits, fatty acid composition,  
40 nutritional value, prediction.

41

### 42 **1. Introduction**

43 The beef industry is made up of a set of links from farmers to consumers, through processors.

44 Each of these intermediaries has specific expectations that are not necessarily consistent with  
45 each other. Among them, most of farmers have mainly expectations in terms of animal  
46 performance. In addition to the type of animal and its age, the carcass payment scale in Europe is  
47 mainly based on 3 parameters: weight, fattening state and conformation. Apart from a few  
48 specifications for which sensory and/or nutritional properties are specific expectations, otherwise,

49 in most cases, these parameters are not taken into account in the remuneration of breeders  
50 (Monteils et al., 2017).

51 In cattle farming, as in most livestock farming, animal feed is the first item of expenditure (50%  
52 to 60% on average). It is therefore in the interests of farmers to select and breed efficient animals,  
53 that is to say, animals that are able to convert efficiently distributed feed into sales products  
54 (Ellies-Oury et al., 2020). This can have consequences on the nutritional and/or sensory  
55 properties of the meat from their carcasses.

56 Meat stakeholders main concern is the market structure and consumers' demands. The quality of  
57 carcasses (in terms of weight, composition or yields for example) is thus an important parameter  
58 for the meat sector, insofar as it determines the payment of the farmer, the remuneration of the  
59 intermediate link and the assurance of an optimized meat quality (i.e. satisfying the sometimes  
60 antagonistic interests of the various stakeholders of the chain ; Dockès et al., 2011).

61 In general, Europeans claim they want to favour food products that are good for their health in  
62 the coming years (Ellies-Oury et al., 2019; Hocquette et al., 2018). Meats have a relatively  
63 homogenous nutritional composition, at least for proteins. However, their content in lipids  
64 (ranging from 1-2% to around 15%; (Li, 2017; Normand et al., 2005)) and in saturated fatty acids  
65 (some of them being assumed to be detrimental to human health; (McAfee et al., 2010)) are quite  
66 variable. These heterogeneous compositions are thus likely to disturb the consumer.

67 The price of meat is the most important purchase criterion for 78% of the French, ahead of taste  
68 quality (46%) (Centre d'études et de Prospective, 2014). Ellies-Oury et al. (2019) specify in a  
69 recent study that 88% of the respondents would be interested in a system that would ensure a  
70 guaranteed level of meat tenderness and meat sensory quality at the time of purchase.  
71 Furthermore, 95% of these people would be willing to pay more for a cut of meat if such a  
72 system is implemented. Prediction models have previously attempted to establish response laws

73 of muscle properties as a function of animal performances at farm or husbandry factors (Conanec  
74 et al., 2019; Ellies-Oury et al., 2020; Ellies-Oury et al., 2019; Hocquette et al., 2017; Soulat et al.,  
75 2018, 2016). Other work has aimed to establish a model for predicting meat quality based on  
76 carcass properties (Ellies-Oury et al., 2020; Ellies-Oury et al., 2019; Gagaoua et al., 2018).  
77 However, in the light of these various studies, it appears that the response laws and prediction  
78 models cannot always been scaled up through experiments. Indeed, for example, concerning  
79 tenderness, Gagaoua et al. (2018) indicate that the best and interesting discriminators were  
80 fattening duration and dry matter intake, whereas, according to Soulat et al. (2018), meat traits  
81 were improved by the genetic of heifers' parents (*i.e.*, calving ease and early muscularity) and  
82 when heifers were slaughtered older. **The aim of this work was thus to determine whether the**  
83 **relations highlighted between the different parameters of interest to the beef sector (animal**  
84 **performance, sensory quantity and nutritional quality) could be validated between**  
85 **experiments.** To do this, the method developed by Ellies-Oury et al. (2016) was applied in a  
86 similar way to the data collected in the present experiment. Briefly, we used the *ClustOfVar*  
87 approach on data established on animals of different breeds from those studied by Ellies-Oury et  
88 al. (2016). Developed to arrange variables into homogeneous clusters, this method allows  
89 dimension reduction and variable selection (Chavent et al., 2011). Thus, this study should make it  
90 possible to show whether the equations proposed by Ellies-Oury et al. (2016) are robust and  
91 generalizable to different types of production. The ultimate objective of this work is, in the long  
92 term, to predict meat qualities and performances based on different animal husbandry practices,  
93 in order to manage these practices and, ultimately, to achieve quality and performance objectives  
94 in line with demand.

95

## 96 **2. Material and Methods**

97  
98 **Animal breeding and slaughtering procedures** respected the French animal protection legislation,  
99 including licensing of experimenters. The French Veterinary Services controlled and approved  
100 the procedures at the slaughterhouse and at experimental facilities.

101

## 102 **2.1 Animal's management and slaughtering**

103

104 All the experimental procedures performed in this study were approved by the Animal Ethics  
105 Committee of INRA-CIRAD-IFREMER (APAFIS#1765-2015091516305 V3).

106 A total of 32 young Charolais bulls ( $313 \pm 29.5$  days old, mean live weight  $482 \pm 25.8$  kg) were  
107 given individually for 6 months a basal diet consisting in 60% roughage (40% of corn silage and  
108 20% of grass silage) and 40% of concentrate (Herbipole, INRAE, 2018). Daily feed intake and  
109 animal's body weight were recorded every 2 weeks. The average daily gain during growth (ADG  
110 during growth) was calculated from birth to weaning (**estimated to 45 kg**) but also during the  
111 growth and the finishing period. The ingested quantities were recorded every two weeks,  
112 allowing to estimate total and averaged ingested quantities of each component of the diet.

113 Animals **were slaughtered in the experimental slaughterhouse of the INRAE Research Centre in**  
114 **which the slaughter rates are more constrained than in a commercial slaughterhouse. That's why**  
115 **animals** were slaughtered in four times (at four different slaughter date) at the same body weight  
116 ( $736 \pm 39.5$  kg). After 24 hours of food deprivation, animals were slaughtered in the experimental  
117 abattoir of the INRAE Research Centre, under standard conditions and in compliance with  
118 French welfare regulations. The carcasses were not electrically stimulated. They were chilled **at**  
119 **2°C in drying chambers during 24 hours**, then stored at 4°C. A pH meter equipped with a glass

120 electrode allowed to verify the ultimate pH 24h *post-mortem*. The aim was to have a pH in the  
121 *Longissimus thoracis* located between 5.7 and 5.9 between the 6th and 7th rib.

122

## 123 **2.2 Animal performances**

124

125 Average daily gain during the finishing period (ADG during the finishing period), finishing feed  
126 conversion efficiency (ADG during the finishing period / dry matter) and finishing consumption  
127 index (dry matter / ADG during the finishing period) were calculated using feed consumption  
128 (expressed as dry matter or energy intake) during the finishing period and **the live weight at the**  
129 **beginning of the finishing period**. Live weight and slaughter age were recorded at the  
130 slaughterhouse. Carcass weight was **then** determined at slaughter. The removal and dissection of  
131 the sixth rib joint allowed to assess its muscle, fat and bone proportions. According to Robelin &  
132 Geay (1975) regression equations, the composition of the carcass (in content and in proportions)  
133 was estimated, by using 1) the sixth rib muscle, fat and bone proportions and 2) the carcass  
134 weight and measurement of the fatty deposits of the 5th quarter (Alberti et al., 2008). **Carcass**  
135 **yield was also determined**.

136 The amounts in vitamin A and E in plasma were determined by HPLC-UV spectrophotometry  
137 adapted to bovine plasma (Scislowski et al., 2005).

138 Total antioxidant status (SAO) in plasma was measured by a method based on the absorbance of  
139 the ABTS<sup>o</sup>+ cation [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] previously described  
140 by (Gobert et al., 2009).

141 Plasma Malondialdehyde (MDA) was determined by HPLC fluorescence as described earlier by  
142 (Agarwal and Chase, 2002).

143 These parameters, evaluated on the blood of the animals, were considered to be relevant to  
144 animal performance, in particular because of the correlation that exists between efficiency and  
145 the antioxidant status of the animal as reported by Chauhan et al. (2016) in lambs and more  
146 generally because dietary antioxidants intake are known to affect growth (Catoni et al., 2008)  
147 Thus, animal performances were characterized by 26 variables (Table 1). Means of each of the 26  
148 variables studied in this experiment are given in Supplementary Data.

149

150

### 151 **2.3 Muscle sampling**

152

153 Samples (around 700 g) of the *Longissimus thoracis* (LT) muscle from the 10th thoracic rib were  
154 collected 1-day *post mortem*. The muscle samples were conditioned and stored differently  
155 according to the further analyses.

156 Samples for sensory evaluation (around 500 g) were cut into steaks, vacuum packed and kept at  
157 4°C for ageing (14 days). Each sample was then frozen and stored at -20°C awaiting sensory  
158 evaluation. A part of the remaining samples (50 g) was frozen in liquid nitrogen and kept at -  
159 80°C until analyses to determine enzyme activities, protein extraction and myosin heavy chain  
160 (MyHC) quantification. Another part of the samples (150g) was cut into pieces (around 0.5 cm  
161 cross-section), ground into fine and homogeneous powder in liquid nitrogen with a mixer mill  
162 (Retch MM 301, Hann Germany) and stored at -80°C until analyses for collagen determination,  
163 fatty acid composition and intramuscular fat content. Samples for one similar analysis were taken  
164 at the same anatomical position from one animal to another.

165

### 166 **2.4. Muscular properties and sensory analysis**



167

168 Total, insoluble and soluble collagen, cross-links (CLs) and proteoglycans (PG) of LT muscles  
169 were measured according to procedure previously described by Dubost et al. (2013a).

170 Myofibrillar proteins from the LT muscles were extracted on ice as previously described by  
171 Picard et al. (1994). The proteins were separated by SDS-PAGE electrophoresis according to  
172 Picard et al. (2011). The proportions of the various myosin heavy chains (MyHC) were quantified  
173 using densitometry with ImageQuant Software 5500 (Amersham Biosciences/GE Healthcare).  
174 MyHC-IIb percentages were totalled with those of MyHC-IIx.

175 Sensory analyses were done at INRAE “Le Magneraud” station. After 14 days of ageing, each  
176 sample was thawed at 4°C over 24 hours. The meat samples were cut into 1.5 thick steaks and  
177 cooked under domestic grills until the temperature **measured with a temperature probe** reached  
178 55°C (typical in France). Steaks were grilled between 2 aluminum sheets, until the end-point  
179 temperature previously indicated in the geometric center of the steak was reached. After grilling,  
180 each steak was cut into portions that were immediately presented to 12 panellists trained in beef  
181 meat sensory analysis. Each sample was rated between 0 to 10 on an unstructured scale for the  
182 following attributes: tenderness, juiciness, overall appreciation, overall odor, overall flavor,  
183 rancid flavor, rancid odor, blood flavor, blood odor, fish flavor, fatty flavor, greasy feel, residues.  
184 The score 0 represented a very low rating of the descriptor (extremely tough, very dry, ...) as  
185 opposed to the score 10 which corresponds to a very high rating (extremely tender, extremely  
186 juicy, ...).

187 **Six** sensory session were organized. At each of these sessions, the 12 panelists evaluated 5 or 6  
188 samples randomly selected. Each sample was assessed by each panelist.

189 The expert panelists were trained in accordance with the ISO standards ISO/TC as described by  
190 Gagaoua et al. (2016). The sessions were carried out in a sensory analysis room equipped with

191 individual booths under artificial red light, in order to reduce the influence of the appearance of  
192 the samples. Each tasting booth was equipped with computer terminal linked to a fileserver  
193 running a sensory software (Fizz, version 2.20h; Biosystemes, Couternon, France) that facilitated  
194 the direct entry of assessor ratings. The variables composing sensory quality are reported in the  
195 table 2. Means of each of these variables studied in this experiment are given in Supplementary  
196 Data.

197

## 198 **2.5. Fatty acids composition**

199

200 Total lipids of LT muscle were extracted according to Folch et al. (1957) and quantified by  
201 gravimetry. The FAs were extracted from total lipids and then converted into methyl esters by  
202 transmethylation using borontrifluoride-methanol (14% solution) according the method of  
203 (Bauchart et al., 2005). Fatty acid methyl ester analysis was performed by GLC using a Peri 2100  
204 chromatography system (Perichrom, Saulx-les-Chartreux, France) fitted with a CP-Sil 88 glass  
205 capillary column (Varian, Palo Alto, CA; length 100 m, dia. 0.25 mm) as previously described by  
206 Gruffat et al. (2020). The carrier gas was H<sub>2</sub>, and the oven and flame ionization detector  
207 temperatures were as follows: oven temperature was programmed for 70°C for 30 s, 70 to 175°C  
208 at a rate of 20°C/min, 175°C for 25 min, then 175 to 215°C at a rate of 10°C/min, and finally  
209 215°C for 41 min; injector and detector temperatures were 235 and 250°C, respectively. Total  
210 FAs were quantified using 19:0 as an internal standard. Their identification and the calculation of  
211 the response coefficients were done using a C4-C24 quantitative mix (Supelco, Bellafonte, PA).  
212 The variables composing nutritional quality are reported in the table 3. Means of each of these  
213 variables studied in this experiment are given in Supplementary Data.

214

## 215 **2.6. Statistical analysis**

216

217 As previously indicated, in order to determine whether **the associations** observed between the  
218 different Parameters of Interest (PI: animal performance, sensory quantity and nutritional  
219 quality) can be scaled up through experiments, we applied the *ClustOfVar* (clustering of  
220 variables; (Chavent et al., 2011)) approach to the data collected in the present experiment for  
221 LT muscle (as this muscle was used in (Ellies-Oury et al., 2016) previous work).

222 Briefly, the proposed statistical methodology splits into 3 steps.

223 First, for each of the three PI, a hierarchical clustering of variables was applied to assess the  
224 links between the variables (within each PI) [step 1, figure 1]. The production of a tree structure  
225 within the variables allows to highlight hierarchical links between variables and to detect an  
226 ideal number of classes within the population (by defining a cut-off level). Clusters, grouping  
227 together the associated variables, were thus created. For each PI and each cluster, an  
228 Intermediate Score (IS) was calculated [step 1], the numerical IS summarizes all the numerical or  
229 categorical variables within the cluster.

230 More precisely, in the *ClustOfVar* method, the IS of a cluster is defined as the first principal  
231 component obtained from the *PCAmix* (principal component analysis for mixed data) method  
232 applied on the variables within the considered cluster. The underlying homogeneity criterion of  
233 a cluster is defined as the sum of correlation ratios (for categorical variables) and squared  
234 Pearson correlations (for numerical variables) to the synthetic numerical variable (the IS in the  
235 proposed statistical methodology), summarizing “as good as possible” all the variables in the  
236 cluster. Thus, a cluster of variables is defined as homogeneous when all the variables in the

237 cluster are strongly linked to the corresponding numerical synthetic variable (the IS). Note that,  
238 more generally, the numerical synthetic variables of the clusters can also be used for dimension  
239 reduction or for recoding purpose. The IS scores reflect all the information contained in the  
240 obtained clusters as long as each cluster is homogeneous. To determine the suitable number of  
241 clusters for a given set of variables, a bootstrap approach has been also developed to evaluate  
242 the stability of the partitions of variables and then provide the best partition (Chavent et al.,  
243 2011). In the following, to interpret the IS, only the variables within the cluster having a squared  
244 correlation with the IS greater than 0.50 were used. Note that the variables selected may be  
245 positively or negatively correlated with the IS.

246 Then, a second *ClustOfVar* analysis was done on all the previously created IS, allowing to build  
247 Global Indexes (GI) that were characterized by the IS's that are the best correlated to these GI  
248 (square correlation greater than 0.50) [step 2, figure 1].

249 Finally, in step 3 [step 2, figure 1], these GI were lastly compared between the two experiments  
250 in order to highlight robust relationships. **That for, variable associations within each GI were**  
251 **compared. Stable pairings from one experiment to another (constituting equivalent clusters)**  
252 **could thus be identified. Conversely, it was also possible to highlight the variables for which the**  
253 **associations were unstable, i.e. the variables that changed cluster between the two experiments.**

254

### 255 **3. RESULTS and DISCUSSION**

#### 256 **3.1. Determination of linked variables in the present experiment**

257

258 The first result is that it was possible to constitute clusters of variables and thus build  
259 Intermediate Scores (IS). In the first step, 5 Intermediate Scores [denoted AP1\* to AP5\*] were

260 retained concerning Animal Performances (figure 2) and respectively 4 and 5 IS concerning  
261 Sensorial [SQ1\* to SQ4\*] (figure 3) and Nutritional Quality [NQ1\* to NQ5\*] (figure 4). The  
262 increase of each of these IS led to either an increase or a decrease of the variables that compose  
263 the cluster, as indicated in table 4.

264 The clusters associated variables known to be correlated, especially because the number of  
265 clusters for each PI is relatively high compared to the total number of variables per PI: 5 vs. 26  
266 for Animal Performances, 4 vs. 20 for Sensory Quality and 5 vs. 57 for Nutritional Quality. As  
267 for Ellies-Oury et al (2016), the synthetic indexes highlighted reflect widely accepted  
268 relationships such as:

- 269 - the positive link between food efficiency and ADG,
- 270 - the positive link between the different sensory parameters,
- 271 - the negative association between PUFA's proportions and the proportions of SFA and MUFA,

272  
273 In the second step, it was possible to build Global Indexes (GI) by making clusters within  
274 Intermediate Scores. Four GI were highlighted [GI1\* to GI4\*] (table 5; figure 5). With the  
275 exception of GI4\*, which associated only ISs related to animal performance [AP2\*, AP3\* and  
276 AP5\*], all other GIs associated ISs related to different PI:

277 - GI1\* associated positively NQ3\*, SQ1\* and SQ4\*. Thus, in this GI, the proportions of  
278 CLA and *trans* MUFA were positively associated with the sensorial descriptors of fatty flavor,  
279 rancid flavor, greasy feel and residues, but also with MyHCIIa isoforms and collagen content  
280 (total, insoluble and CLs contents). All these parameters were negatively linked to tenderness.

281 - GI2\* associated positively AP1\* and negatively NQ1\*, NQ2\*, NQ4\* and SQ3\*. The  
282 SQ2\* Intermediate Score was also negatively associated in this GI, but as the square correlation  
283 was lower than 0.50, this IS was removed for the GI2\* interpretation. In this GI, the proportions

284 of PUFA were positively associated with live and carcass weights but also with rancid and fish  
285 flavors. All these variables were negatively linked to the amounts of lipids, the amounts and  
286 proportions of SFA and MUFA, but also to the positive descriptors of juiciness, overall flavor,  
287 overall odor and overall appreciation.

288 - GI3\* associates positively AP4\* and NQ5\*. According to the interrelations identified in  
289 this GI, the amounts of PUFA were positively associated with slaughter age and ADG between  
290 birth and weaning.

291 To finish with, the GI4\* associated ingested quantities, feed efficiency, carcass yield and muscle  
292 development, finishing growth rate and MDA content.

293

294 As noted earlier, the *ClustOfVar* method allows to group together variables that share similar  
295 information and that are thus highly correlated to each other. The advantage of this method is that,  
296 in the same analysis, different types of variables (contents, proportions, ratios, ...) can be grouped  
297 together, without any problem of collinearity. This specificity of the clustering of variables is  
298 clearly useful in our case, in that some of the different variables used are in part redundant with  
299 each other. On the other hand, in the method used here, two successive clustering of variables  
300 were carried out (steps 1 and 2) in order to compare our results with those established by Ellies-  
301 Oury et al. (2016). This succession of clustering is at the origin of a loss of information that is  
302 likely to limit the identification of relationships between the studied variables all the more as, as  
303 previously indicated, only the variables having a square correlation with the IS greater than 0.50  
304 were used.

305 The variable clustering approach aims to maximize a homogeneity criterion among the highly  
306 correlated variables. However, from one experiment to another one, the available variables might  
307 be different and the homogeneity criterion might thus be different.

308 To finish with, what might also be underlined, the stability of partitions of variables was  
309 evaluated with bootstrap approach (Chavent et al., 2011). However, other methods might have  
310 been developed to determine the suitable number of clusters of variables, and might have  
311 conducted to a number of clusters different, and thus to different associations between variables.  
312 Nevertheless, the evaluation of the associated variables under other conditions of determination  
313 of the number of clusters makes it possible to highlight that the general tendencies remain  
314 unchanged whatever the number of clusters finally formed.

315  
316 In the light of the associations of variables found in this paper, it can be shown that the main  
317 conclusions previously established by Ellies-Oury et al. (2016) on the LT muscle of Angus,  
318 Blondes d'Aquitaine and Limousin bulls were also true in this new experiment on the same  
319 muscle collected on Charolais bulls.

320  
321 For example, we established that juiciness and the descriptors of overall appreciation, overall  
322 flavor and overall odor were positively related to meat lipid content but negatively related to the  
323 proportions of PUFA and EPA. Moreover, it appears that the positive sensory descriptors of  
324 juiciness as well as the overall odor, flavor and appreciation descriptors were negatively  
325 associated to carcass weight and ADG during growth. ~~It can be hypothesized that this negative~~  
326 ~~association is related to lower fat development of animals with a high ADG during growth, as~~  
327 ~~already established by Ellies-Oury et al. (2016). Indeed, during growth, most of nutrients are used~~  
328 ~~for muscle development instead of fat development and thus, intramuscular fat (IMF) is deposited~~  
329 ~~at a lower rate than muscle proteins. On the contrary, IMF is deposited at a greater rate than~~  
330 ~~muscle proteins during finishing period, since less nutrients are used for muscle growth (Pethick~~  
331 ~~et al., 2004).~~

332 Positive sensory descriptors of juiciness, overall appreciation, overall flavor and overall odor  
333 were also negatively associated with nutritional value of meat. As described above, increasing  
334 lipid content in meat led **in most publications** to an increase in sensorial descriptors of tenderness,  
335 juiciness, flavor and overall acceptability scores in trained jury but also in consumer sensory  
336 panels (Garmyn et al., 2011; Hocquette et al., 2010; Jeremiah et al., 2003; Lorenzen et al., 2003;  
337 May et al., 1992; Mottram, 1998; Neely et al., 1998; O'Quinn et al., 2012; Savell et al., 1987;  
338 Smith et al., 1985). However, even if fat and fatty acids contribute to a large extent to meat  
339 sensory quality, they also contribute to the nutritional value of meat (Wood et al., 2008; Scollan  
340 et al., 2001, 2014; Wyness et al., 2011). Indeed, for human, the relationships between dietary fat,  
341 irrespective of the food source, with the incidence of various lifestyle diseases including  
342 cardiovascular diseases is well established and several health agencies have proposed specific  
343 guidelines. Some fatty acids are also known to affect human health in several ways, especially  
344 some SFAs, such as C16:0, that is known for their harmful properties on human health  
345 (Muchenje et al., 2009). In the case of beef, the fatty acids present in the adipocytes being mostly  
346 SFAs and MUFAs (Wood et al., 2008), fatter animals have higher SFA proportions in their  
347 muscles and, thus, deleterious fatty acids.

348 However, juiciness, flavor/odor and overall appreciations are positively related to amounts and  
349 proportions of C18:1, confirming by the same way, that the fatter the animals are, the higher the  
350 proportion of SFA and MUFA (and therefore of C18:1 which is the major fatty acid in MUFA) in  
351 the meat, the juicier the meat. These results confirm the conclusions of various studies (Dryden  
352 and Maechello, 1970; Garmyn et al., 2011; Melton et al., 1982; Westerling and Hedrick, 1979),  
353 that establish a positive correlation between beef palatability and C18:1. Moreover, this FA is  
354 known to be a "neutral" FA without any deleterious or beneficial impact on human health  
355 (Pereira and Vicente, 2013).



356 ~~In addition, as previously indicated by Ellies Oury et al. (2016), juiciness and the descriptors of~~  
357 ~~overall appreciation, overall flavor and overall odor were positively related to meat lipid content~~  
358 ~~but negatively related to the proportions of PUFA and EPA. This result is logical since, as~~  
359 ~~explained above, the increase in lipids in meat leads to an increase in the proportions of SFA and~~  
360 ~~MUFA and thus a decrease in the proportion of PUFA. PUFAs are widely recognized for their~~  
361 ~~beneficial properties for human health, including EPA and DHA which can profoundly influence~~  
362 ~~human health (Vahmani et al., 2015). Thus, once again, juiciness and the descriptors of overall~~  
363 ~~appreciation, overall flavor and overall odor are negatively correlated with the health value of~~  
364 ~~meat.~~ Surprisingly, the tenderness is negatively related to the proportions of CLA and CLA  
365 precursors. These FA are known to reduce body fat, cardiovascular diseases and cancer, and to  
366 modulate immune and inflammatory responses (Dilzer and Park, 2012; Whigham et al., 2000).  
367 This negative **relationship** is surprising since CLAs are mainly stored in triglycerides (that are  
368 storage lipids that increase with body fat). According to the literature, tenderness might also be  
369 negatively correlated to PUFA proportions, whereas its correlation with MUFA appears positive  
370 (Garmyn et al., 2011). In conclusion, it seems complicated to jointly obtain a meat with  
371 optimized sensory and nutritional properties. There is a need to understand the relationship  
372 between palatability, fatty acid composition and fat content, in order to ensure subsequently that  
373 tenderness, flavor, and juiciness are not compromised when selecting cattle with enhanced  
374 nutritional composition.

375 In the present work, n-3 and n-6 PUFA proportions were positively linked (and negatively linked  
376 to n-3 and n-6 PUFA amounts, these amounts being positively linked together). These  
377 conclusions are contrary to those of the literature as in some studies, increasing n-3 PUFA  
378 proportions matched with decreasing n-6 PUFA proportions, showing competition between these  
379 fatty acid families for the same set of elongation and desaturation enzymes (Lorenz et al., 2002;

380 Nuernberg et al., 2005). ~~Working on various bovine breeds, (Ellies Oury et al., 2016) indicated~~  
381 ~~that increasing n-3 PUFA proportions leads to an increase of n-6 PUFA proportions (n-3 PUFA~~  
382 ~~amounts are also positively correlated with n-6 PUFA amounts), but this correlation was found to~~  
383 ~~be breed dependent. In fact, in the literature, most studies compared diets rich in n-3 PUFA to~~  
384 ~~diets rich in n-6 PUFA. It is then normal that an increase in one family of fatty acids is associated~~  
385 ~~with a decrease in the other. In the present experiment, the animals were all fed the same diet~~  
386 ~~(40% of corn silage, 20% of grass silage and 40% of concentrates) which provided both n-6 (corn~~  
387 ~~and concentrates) and n-3 (grass silage) PUFA. Thus, the present results might stem from an~~  
388 ~~animal diet impact, as it is widely accepted that cattle feed (enriched or not with certain PUFAs~~  
389 ~~and in particular n-3 PUFA) might have a significant impact on the FA composition of the meat.~~  
390 ~~If (Ellies Oury et al., 2016) has previously revealed a positive relation between collagen content~~  
391 ~~and lipid content of beef, a link might here be established between collagen and cross link~~  
392 ~~contents and fat linked sensory descriptors such as fat flavor and greasy feel. All of these~~  
393 ~~variables are also positively associated with the proportions of CLA and CLA precursors.~~  
394 ~~Collagen composition (total and insoluble collagen, CLs) was positively associated with residues~~  
395 ~~and negatively with tenderness. This conclusion, which is the opposite of that of (Ellies Oury et~~  
396 ~~al., 2016), is however consistent with the results previously established in the literature, which~~  
397 ~~associated the increase in collagen content with an increase in meat hardness (Destefanis et al.,~~  
398 ~~2000; Dransfield et al., 2003; Dubost et al., 2013b; Lepetit, 2007; McCormick, 1999; Nishimura~~  
399 ~~et al., 2009; Renand et al., 2001; Rhee et al., 2004; Torrecano et al., 2003).~~  
400 No relationship was found between the proportions of the different myosin isoforms and the  
401 sensory quality of the meat, contrary to the results of the literature (Chriki et al., 2013; Crouse et  
402 al., 1991; Ellies-Oury et al., 2016; Jurie et al., 2007; Maltin et al., 1998; Therkildsen et al., 2002;  
403 Zamora et al., 1996), who established a positive association between oxidative metabolism and

404 sensory quality. Nevertheless, the relationship appears controversial as other authors, such as  
405 (Picard et al., 2014), that have found a negative correlation between these same variables,  
406 depending on the muscle type and/or the breed of the animals. Thus, even though several studies  
407 reviewed by Guillemin et al. (2009) have previously considered that fiber metabolic and enzyme  
408 activities are notably implicated in meat quality traits and especially in tenderness, no significant  
409 interrelation are observed in the present work.

410 To finish, we logically establish that carcass butcher value (carcass yield and muscle  
411 development in the carcass) is associated with lower carcass fatness but positively with  
412 peroxidation intensity. As indicated above, the leaner the meat, the more PUFA proportions it  
413 contains. However, it is well known that the PUFAs present in beef are sensitive to oxidation  
414 especially as the level of antioxidants such as vitamins E and A is low (Durand et al., 2005). Thus,  
415 it is not surprising that carcass butcher value is positively associated with MDA levels, that is an  
416 index of peroxidation intensity and negatively associated with the total antioxidant status and  
417 vitamin A and E levels of meat, that are indicators of the protection level of PUFA against  
418 peroxidation (Durand et al., 2005). It would therefore be relevant to introduce antioxidants in the  
419 diet of animals with a high carcass butcher value, in order to maintain the nutritional value of  
420 their meat.

421

#### 422 **4. CONCLUSION**

423 The objective of this work was to determine whether the relations highlighted between the  
424 different parameters of interest to the industry (animal performance, sensory quantity and  
425 nutritional quality) could be validated between experiments. In the present work, we used the  
426 *ClustOfVar* package to validate, with data from a new experiment, the relationships previously  
427 highlighted in the literature (Ellies-Oury et al., 2016). In the light of these associations of

428 variables, it can be shown that the main conclusions previously established on the LT muscles (of  
429 Angus, Blonde d'Aquitaine and Limousine cattle), were also found in this new experiment on the  
430 same muscle but on a different breed (Charolais). Indeed, the generalization of the equations  
431 established between the Parameters of Interest can help to determine the compromises that it will  
432 be necessary to make in order to meet the expectations of the different links of the sector. It will  
433 thus be possible to propose "realistic" specifications adapted to the different breeders / operators /  
434 consumers. However, the generalizations have only been shown to apply for the *Longissimus*  
435 *thoracis* from young bulls finished under feedlot conditions and slaughtered when they achieved  
436 a set weight.

437  
438 In order to widely generalize the established relationships, it is now appropriate to apply this  
439 method to a database containing different experiments with various characteristics. It will thus be  
440 possible to highlight robust and generalizable relationships in different contexts (different breeds,  
441 types of animals, ...).

442 This method was carried out until now on the LT muscle, which is commonly considered as the  
443 reference muscle. However, in the light of various studies, it appears that the response laws and  
444 prediction models cannot always be scaled up through muscles within the same experiment.  
445 Thus, now that it has been proven that the relationships between the Parameters of Interest  
446 (animal performance, sensory quality, nutritional quality) can be transposed from one experiment  
447 to another on the same muscle, it is necessary to determine whether the proximities established  
448 between the three Parameters of Interest could be extrapolated from one muscle to another within  
449 the same carcass. It would then be possible to predict the properties of all the muscles of the  
450 carcass by knowing the properties of one of them. ~~Such predictive equations of the nutritional  
451 and sensory properties of the various muscles of the carcass would be of real interest to the~~

452 ~~industry. Thus, in the long run, it should be possible to integrate these equations into a~~  
453 ~~mathematical model for the management of animals in order to obtain meats whose properties~~  
454 ~~will perfectly meet the expectations of the different markets / market niches.~~

455

#### 456 Funding

457 This study was funded by ADEME (Agence de l'Environnement et de la Maîtrise de l'Energie,  
458 France).

459

#### 460 Acknowledgments

461 The authors express their thanks to the staff of the Studyal Unit, Herbipôle (UERT) of INRAE  
462 (Theix) for the animal management, slaughtering and the staff of Biomarkers team for their  
463 skilled technical assistance, particularly Arnaud Delavaud, David Chadeyron, Blandine Fréchet  
464 for the collagen, cross-link and proteoglycans content measurements and Agnès Thomas, Sylvie  
465 Bardou-Valette and Mélissa Vauris for the muscle lipid, fatty acids, peroxidation and antioxidant  
466 determinations. The authors also express their thanks to the staff and the panelists of the INRAE  
467 Unit (Le Magneraud) for the sensory quality evaluations. We also thank Emmanuel Bedier and  
468 Mathieu Pondet from Idena and Bérengère Hoes from Thivat Nutrition Animale, who allowed  
469 this project happen.

470

471

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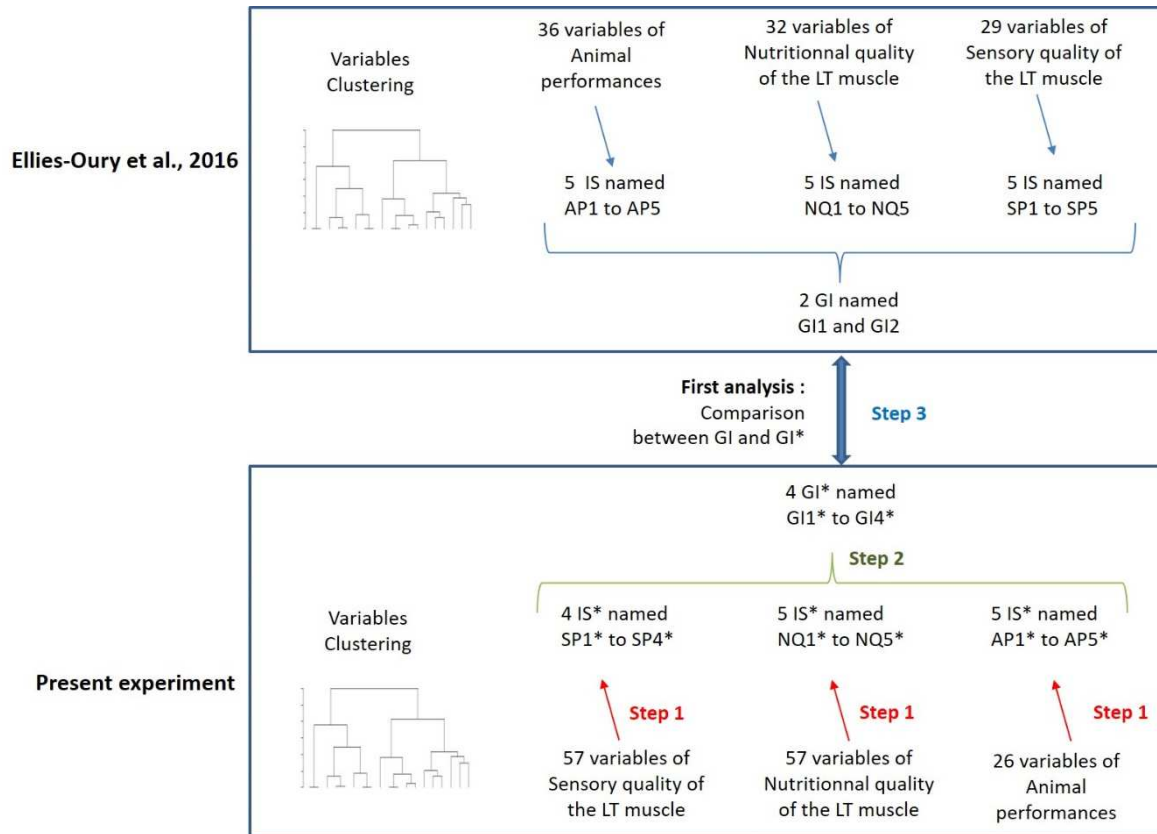


Figure 1: principle of the method developed by Ellies-Oury et al. (2016) and in the present experiment  
 IS: intermediate score – GI: global index – LT: *Longissimus thoracis* – AP: animal performance – NQ: nutritional quality – SQ: sensory quality

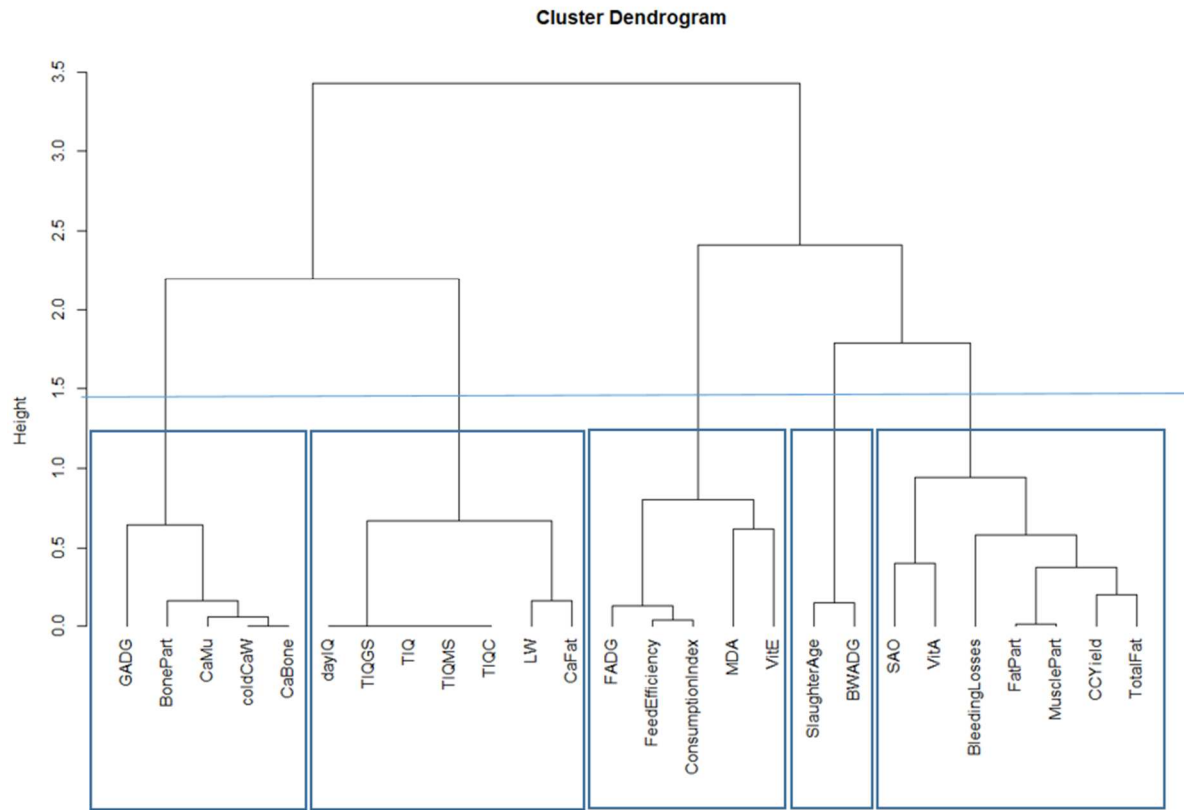
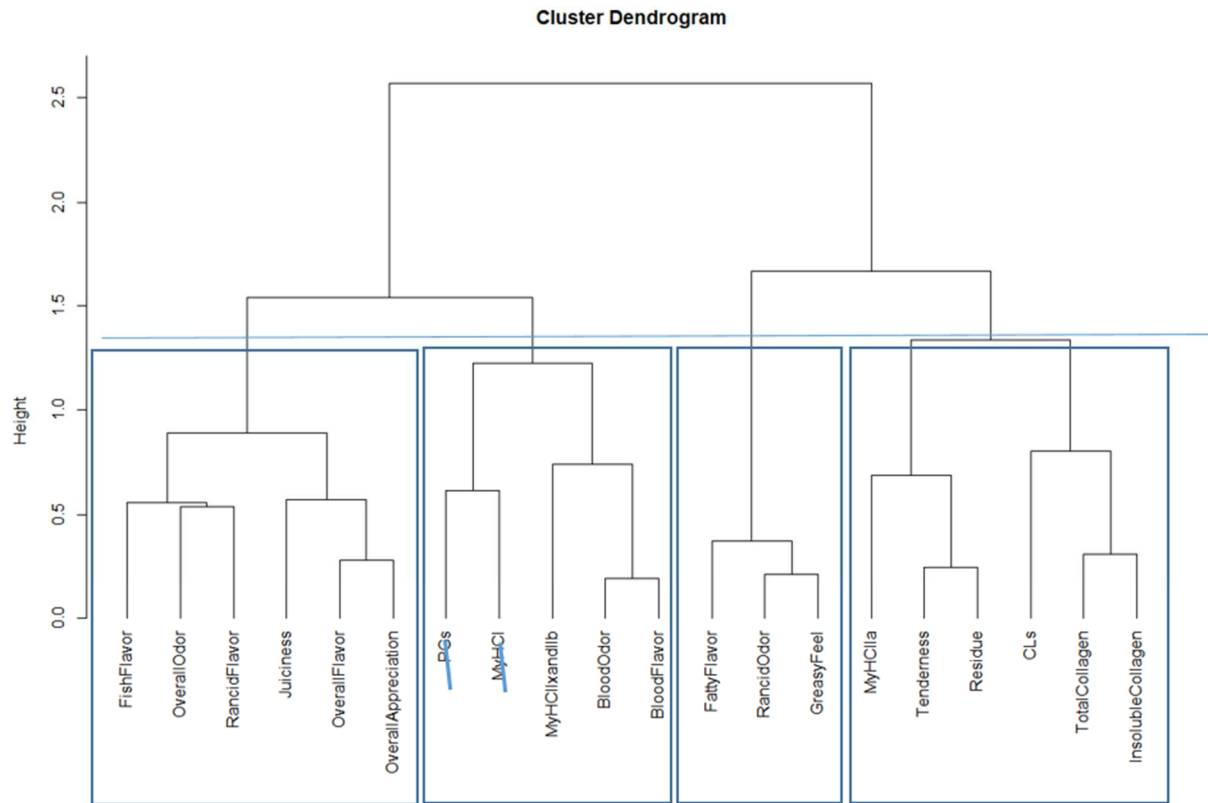


Figure 2: Intermediate Scores (n=5) retained concerning Animal Performances



**Figure 3: Intermediate Scores (n=4) retained concerning Sensorial Quality**

*The 2 crossed-out variables (PGs, MyHCI) correspond to variables poorly represented in the cluster (and not analyzed in the following).*

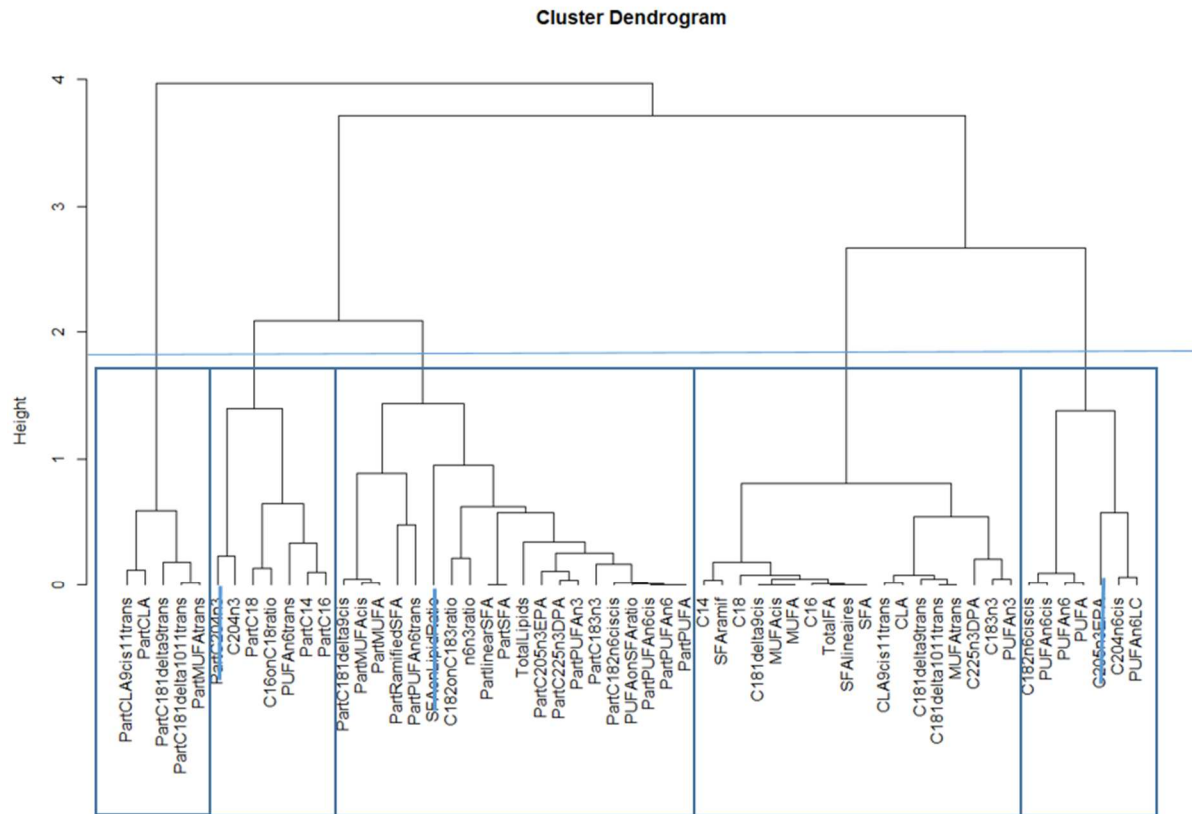


Figure 4: Intermediate Scores (n=5) retained concerning Nutritional Quality  
 The 3 crossed-out variables (%C20:4n3; SFA/Lipid ratio; C20:5n3EPA) correspond to variables poorly represented in the cluster (and not analyzed in the following)

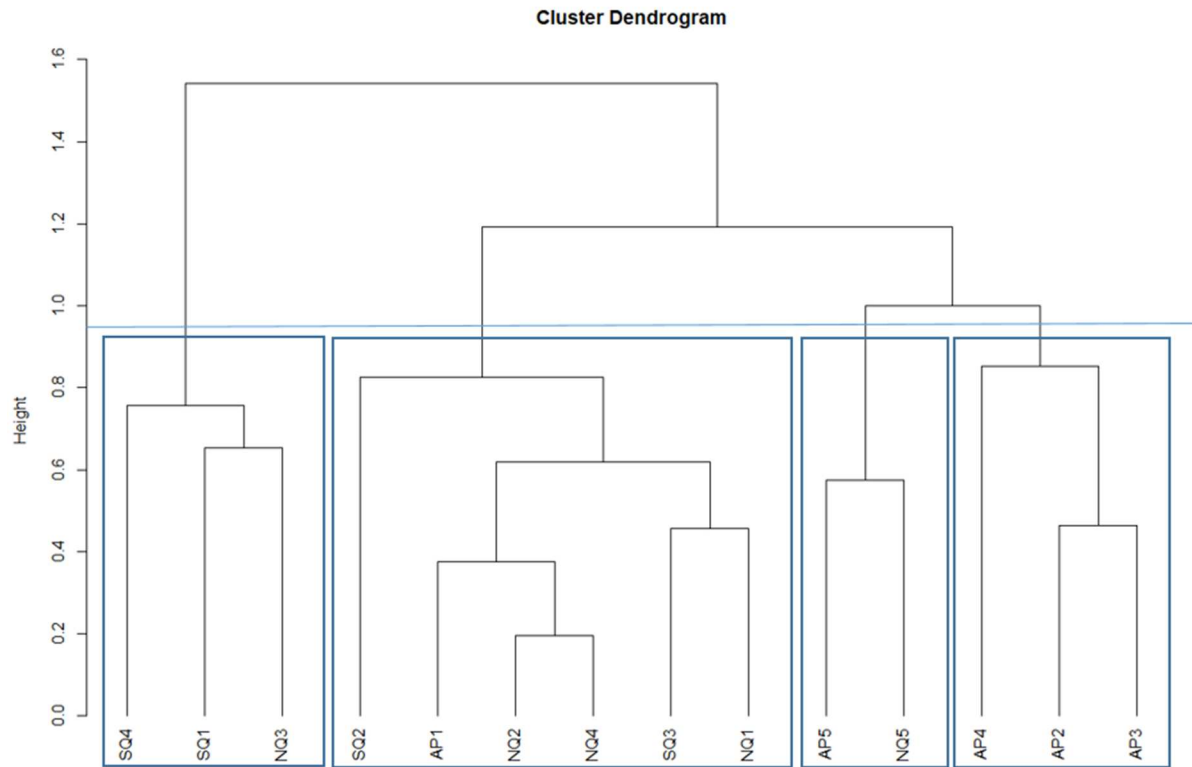


Figure 5: Global Indexes (GI) built by making clusters within Intermediate Scores  
 5 Intermediate Scores [denoted AP1\* to AP5\*] concerning Animal Performances  
 4 IS concerning Sensorial Quality [SQ1\* to SQ4\*]  
 5 IS concerning Nutritional Quality [NQ1\* to NQ5\*]

Table 1: list of the 26 variables of animal performances analyzed on the 32 young Charolais bulls

Variables
Age at slaughter; Total ingested quantities in grass silage (TIQGS), maize silage (TIQMS), concentrate (TIQC); Total ingested quantities (TIQ); Ingested quantities per day (dayIQ);
Live weight (LW); Carcass weight (CaW);
Carcass Yield (CCYield = $CaW / LW$ ); Bleeding losses; Total fat weight (Total Fat);
Carcass fat weight (CaFat); Carcass proportion of fat (FatPart);
Carcass bone weight (CaBone); Carcass proportion of bone (BonePart);
Carcass muscle weight (CaMuscle); Carcass proportion of muscle (MusclePart);
Average Daily Gain (ADG) between birth and weaning (BWADG), during growth (GADG), and during finishing period (FADG)
Feed conversion Efficiency (ADG / total dry matter intake); Consumption index (total dry matter intake / ADG)
Amount in A and E vitamins;
MDA; SAO



Table 2: list of the 20 variables of sensory quality analyzed on the 32 young Charolais bulls

Variables
Tenderness; Juiciness; Residue; Overall appreciation; Overall odor; Overall Flavor; Fish flavor; Blood odor; Blood flavor; Rancid odor; Rancid flavor; Fatty flavor; Greasy feel; Total collagen; Insoluble collagen; CLs; PGs; MyHC I; MyHC IIa ; MyHC IIx+b;

Table 3: list of the 57 variables of nutritional quality analyzed on the 32 young Charolais bulls

Variables
<p><b>Fatty acid composition expressed in content (g or mg / 100 g of muscle):</b></p> <p>Total Lipids; Total Fatty Acids (FA); Saturated FA (SFA); Monounsaturated FA (MUFA); Polyunsaturated FA (PUFA); C14:0; C16:0; C18:0; ramified SFA; linear SFA; C18:1 n-9 <i>cis</i>; <i>trans</i> C18:1 10/11; <i>trans</i> C18:1 n-9; <i>cis</i> MUFA; <i>trans</i> MUFA; C18:2 n-6; <i>cis</i> C20:4 n-6; PUFA n-6; C18:3 n-3; DPA; EPA; PUFA n-3; long chain PUFA n-3; Total Conjugated Linoleic Acids (CLA); CLA <i>9cis</i> <i>11trans</i>; <i>trans</i> PUFA; <i>cis</i> PUFA</p> <p><b>Fatty acid composition expressed in proportions (% of total FA):</b></p> <p>Total Fatty Acids; Saturated FA; Monounsaturated FA; Polyunsaturated FA; C14:0; C16:0; C18:0; ramified SFA; linear SFA; C18:1 n-9 <i>cis</i>; C18:1 <i>9trans</i>; C18:1 10/11<i>trans</i>; <i>cis</i> MUFA; <i>trans</i> MUFA; C18:2 n-6; C20:4 n-6; PUFA n-6; <i>trans</i> PUFA n-6; <i>cis</i> PUFA n-6; C18:3 n-3; EPA; DPA; PUFA n-3; Total Conjugated Linoleic Acids; CLA <i>9cis</i> <i>11trans</i></p> <p><b>Ratios :</b> C16:0 / C18:0; PUFA / SFA; PUFA n-6 / PUFA n-3 ; C18:2 n-6 / C18:3 n-3</p>

Table 4: Composition of each of the Intermediate Scores obtained for each parameter of interest (animal performances, sensorial quality and nutritional quality) in both experiment

	Ellies-Oury et al., 2016	Present experiment
Animal Performances (AP)	AP1 Fat development in the carcass and in the 5 <sup>th</sup> quarter, but also total fat and trim fat	AP1* Carcass Weight, Amount of muscles/bone in the carcass, ADG during growth <b>Negatively linked to Part of Bone in the carcass</b>
	AP2 Empty body weight and bone development	AP2* Bleeding losses, Carcass yield, Part of muscle in the carcass, <b>Negatively linked to Fat development in the carcass, amount in A vitamins, Part of fat in the carcass, SAO</b>
	AP3 Feed conversion efficiency, ADG during finishing period	AP3* Feed conversion efficiency, ADG during finishing period, MDA <b>Negatively linked to Consumption index, amount in E vitamins</b>
	AP4 Viscera weight and proportions	AP4* <b>Live weight, Amount of fat in the carcass, ingested quantities (total, per day, GS, MS, C)</b>
	AP5 Butcher value (compactness, thigh thickness, muscle development, yields, carcass weight)	AP5* Age and ADG between birth and weaning
Sensorial Quality (SQ)	SQ1 Meat quality traits of steaks cooked at 55°C (higher tenderness and lower residue content) and/or 74°C (higher tenderness and juiciness and lower residue content)	SQ1* MyHCIIA, Residues, collagen amounts (total, insoluble) and CLs <b>Negatively linked to Tenderness</b>
	SQ2 Whiteness (L*) <b>Negatively linked to yellowness (b*)</b>	SQ2* MyHC IIX and IIB, blood odor and flavor
	SQ3 Oxidative metabolism (ICDH and COX activities and %MyHC I)	SQ3* Juiciness and Overall flavor/odor/appreciation <b>Negatively linked to Rancid and fish flavors</b>
	SQ4 Collagen content and insolubility, lipid content <b>Negatively linked to glycolytic fibres proportions (MyHC IIx+IIb)</b>	SQ4* Fatty flavor, rancid odor and greasy feel
	SQ5 Protein content	
Nutritional Quality (NQ)	NQ1 n-6/n-3 ratios (n-6/n-3 and C18:2 n-6 / C18: 3n-3)	NQ1* C16:0/C18:0, % C14:0, % C16:0, <i>trans</i> PUFA amount <b>Negatively linked to % C18:0, % C20:4 n-3</b>
	NQ2 Total lipids, Amounts of SFA, CLA and MUFA	NQ2* Lipids, % SFA, % linear SFA, % MUFA, % <i>cis</i> MUFA, % <i>cis</i> C18:1D9 <b>Negatively linked to C18:2 n-6/C18:3 n-3, n6/n3, % ramified SFA, PUFA/SFA % EPA, % DPA, % n-3 PUFA, % C18:2 n-6 <i>cis cis</i>, % C18:3 n-3, % <i>trans</i> n-6 PUFA, % <i>cis</i> n-6 PUFA, % n-6 PUFA, %PUFA</b>
	NQ3 % MUFA <i>trans</i>	NQ3* % CLA, % CLA 9 <i>cis</i> 11 <i>trans</i> , % C18:1 9 <i>trans</i> , % C18:1 10-11 <i>trans</i> , % <i>trans</i> MUFA
	NQ4 % PUFA, % n-6 PUFA, % n-3 PUFA <b>Negatively linked to %SFA and %MUFA</b>	NQ4* Amounts of FA, C14:0, C16:0, C18:0, ramified SFA, linear SFA, SFA, <i>cis</i> MUFA, MUFA, C18:1n-9 <i>cis</i> , CLA 9 <i>cis</i> 11 <i>trans</i> , CLA, C18:1 9 <i>trans</i> , C18:1 10-11 <i>trans</i> , <i>trans</i> MUFA, C22:5 n-3 DPA, C18:3 n-3, n-3 PUFA
	NQ5	NQ5*

	Amounts of PUFA, n-3 PUFA and n-6 PUFA	Amounts of PUFA, n-6 PUFA, long chain n-6 PUFA, <i>cis</i> n-6 PUFA, C18:2 n-6 <i>cis cis</i> , C20:4 n-6 <i>cis</i>
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Table 5: Composition of each Global Index established by a clustering of the previous IS in both experiment

Ellies-Oury et al., 2016	Present experiment
<p><b>GI1: + AP1 + NQ1 - SQ1</b> Butcher value (compactness, thigh thickness, muscle development, yields, carcass weight), % PUFA, % PUFA n-3, % PUFA n-6, Glycolytic fibres, Residues <b>Negatively linked to</b> Fat development, Non-productive needs, Lipid, SFA, MUFA, PUFA, n-3 PUFA, n-6 PUFA, % SFA, % C14:0, % C16:0, % MUFA, Tenderness (55°C and 74°C), Juiciness (74°C), Oxidative metabolism, Collagen amounts (total, insoluble)</p>	<p><b>GI 1*: + NQ3* + SQ1* + SQ4*</b> % CLA, % CLA 9cis 11trans, % C18 :1 9trans, % C18 :1 10-11trans, % trans MUFA MyHCIIA, Residues, collagen amounts (total, insoluble) and CLs Fatty flavor, rancid odor and greasy feel <b>Negatively linked to</b> Tenderness</p>
<p><b>GI2 = + NQ2 + SQ2</b> % MUFA trans, Yellowness (b*), Protein content <b>Negatively linked to</b> Whiteness (L*), n-6 / n-3, C18:2 n-6/C18:3 n-3</p>	<p><b>GI 2 *: + AP1* - NQ2* - NQ4* - NQ1* - SQ3* (-SQ2*)</b> Carcass weight, amount of muscles/bone in the carcass, ADG during growth % C18:0, % C20:4 n-3, % EPA, % DPA, %, n-3 PUFA, % C18:2 n-6 cis cis, % n-6 PUFA, %PUFA, % C18:3 n-3, % ramified SFA, % cis n-6 PUFA, % trans n-6 PUFA, C18:2 n-6/C18:3 n-3, n-6/n-3, PUFA/SFA, Rancid and fish flavors <b>Negatively linked to</b> C16:0/C18:0, Lipids % SFA, % C14:0, % C16:0, % linear SFA, % MUFA, % cis MUFA, % C18:1 n-9 cis; Amount of FA, C14:0, C16:0, C18:0, ramified SFA, linear SFA, SFA, cis MUFA, MUFA, C18:1 n-9 cis, CLA 9 cis 11 trans, CLA, C18:1 9trans, C18:1 10-11trans, trans MUFA, C22:5 n-3, C18:3 n-3, n-3 PUFA, trans PUFA, Juiciness and Overall flavor/odor/appreciation, <b>Part of Bone in the carcass</b></p> <p><b>GI 3*: + NQ5* + AP4*</b> Amount of PUFA, n-6 PUFA, long chain n-6 PUFA, cis n-6 PUFA, C18:2 n-6 cis cis, C20:4 n-6 cis Age and ADG between birth and weaning</p> <p><b>GI 4*: + AP3* + AP2* + AP5*</b> <b>Live weight, amount of fat in the carcass</b>, Ingested quantities, Bleeding losses, Carcass yield, Part of muscle in the carcass, Feed conversion efficiency, ADG during finishing period, MDA <b>Negatively linked to</b> Fat development in the carcass, Part of fat in the carcass, Consumption index, Amount in A and E vitamins, SAO</p>