

1 **Relationships of a Detailed Mineral Profile of Meat with Animal Performance and Beef**  
2 **Quality**

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### 30 **Summary**

31 Minerals play direct or indirect role in different biological process of animals. These biological  
32 processes finally affect the meat qualities. Therefore, analysis of minerals in livestock is important  
33 for assessing the meat quality and their relation or potential effects on beef quality. However,  
34 minerals profile and concentration in meat are affected by several factors such as animals rearing  
35 practices, age, environment, breed etc. Therefore, we have analyzed 20 minerals in 192 beef  
36 samples and studied the different sources of variation which affect the minerals profile in beef. In  
37 order to understand the complex and intriguing relations of meat qualities and minerals, we have  
38 utilized correlation and factor analysis with 16 traits related to animal performance and meat quality.  
39 Our analysis shows that indeed there are many significant associations of minerals in beef with  
40 animal performance and meat qualities. Five groups of minerals (latent factors) were associated  
41 with almost all quality traits of beef. The knowledge about the mineral contents in beef is important  
42 to understand the complex interrelationships of animal rearing, farm management, environmental  
43 conditions with regard to animal performance and meat quality.

### 44 **Abstract**

45 Mineral profile of beef interests human health, but also animal performance and meat quality. This  
46 study analyzes the relationships of 20 minerals in beef (ICP-OES) with 3 animal performance and  
47 13 meat quality traits analyzed on 182 samples of *Longissimus thoracis*. Animals' breed and sex  
48 showed limited effects. The major sources of variation (farm/date of slaughter, individual animal  
49 within group and side/sample within animal) differed greatly from trait to trait. Mineral contents  
50 were correlated to animal performance and meat quality being significant 52 out of the 320  
51 correlations at the farm/date level, and 101 out of the 320 at the individual animal level. Five latent

52 factors explained 69% of mineral co-variation. The most important, “*Mineral quantity*” factor  
53 correlated with age at slaughter and with the meat color traits. Two latent factors (“*Na+Fe+Cu*”  
54 and “*Fe+Mn*”) correlated with performance and meat color traits. Two other (“*K-B-Pb*” and “*Zn*”)  
55 correlated with meat chemical composition and the latter also with carcass weight and daily gain,  
56 and meat color traits. Meat cooking losses correlated with “*K-B-Pb*”. Latent factor analysis appears  
57 be a useful means of disentangling the very complex relationships that the minerals in meat have  
58 with animal performance and meat quality traits.

59

60 Keywords: macro-minerals; micro-minerals; environmental-minerals; beef quality; beef production;  
61 multivariate analysis

62

## 63 1. Introduction

64 Minerals are valuable nutrients and essential for both human and animal health [1]. Minerals  
65 are known to have a large influence on human health, effecting a wide variety of body functions, for  
66 example, enzyme function, osmotic pressure control, muscle contraction, etc.[2–4]. Minerals also  
67 plays crucial role in meat quality because they affect the several biological process in animals and  
68 some characteristics of meat such as color and texture. Therefore, minerals are essential, so their  
69 characterization in meat and exploring their relation with animal performance and beef quality  
70 becomes important. However, as several studies have shown that type and concentrations of  
71 minerals in meat are affected by several factors, such as the animal's breed, sex, age, diet and water  
72 intake, farm management system, and environmental conditions [5–7], and muscle type and  
73 cooking procedure [8]. The mineral content of meat has been studied in particular in relation to its  
74 value as a nutrient for humans [9,10] and, although less frequently, in relation to the adequacy of  
75 the animals' dietary mineral supply [1]. Although several studies have quantified the minerals  
76 contained in beef, most have looked at only a few of them - macro-minerals and some essential  
77 micro-minerals - and have often sampled only one experimental farm with one feeding regime.

78 Furthermore, with a few exceptions, such as the relationships between iron content and meat  
79 color [11], and between calcium content and meat tenderness [12,13], almost no studies have  
80 systematically analyzed the relationships between mineral content and beef quality traits.

81 As we analyzed fairly large numbers of minerals, productive and qualitative traits, we did not set  
82 out to examine in detail and discuss each single mineral and its relationships with all the animal and  
83 meat traits, but rather aimed to obtain a general picture of the main associations between the  
84 detailed mineral profile and animal performance and meat quality. The specific objectives of this  
85 investigation, therefore, were: a) to analyze firstly the sources of variation in animal performance  
86 and beef quality traits (breed, sex, farm, animal within farm, sample within animal) in animals  
87 reared on 15 different farms in accordance with a recognized beef production system, which we  
88 treated as a case study; b) to analyze the relationships between 20 mineral concentrations in beef (6

89 macro-minerals, 5 essential micro-minerals, and 9 environmental micro-minerals) and animal  
90 performance and beef quality traits at the farm level and also at the level of individual animal within  
91 farm; and c) to analyze the relationships of the latent explanatory factors condensing the major part  
92 of the co-variation in the minerals with animal performance and meat quality as a means of  
93 identifying possible pathways characterizing the complex picture emerging from the correlations.

## 94 **2. Materials and methods**

### 95 *2.1. Farms and animals*

96 The beef production system that serves as our case study has Protected Geographical  
97 Indication (PGI) certification under European Union regulation 134/1998 with the designation  
98 “Vitellone Bianco dell’Appennino Centrale” (Central Apennine White Young Bull). Fifteen farms  
99 in the historical areas of origin of the Chianina (mainly Tuscany/Umbria) and the Romagnola  
100 (Emilia-Romagna) breeds, were selected by the “Consorzio Produttori Carne Bovina Pregiata delle  
101 Razze Italiane” (CCBI, Consortium of Producers of High-Quality Beef from Italian Breeds), which  
102 is responsible for controlling and monitoring the PGI certification. Ninety-one young bulls and  
103 heifers of the two breeds were randomly sampled from the 15 selected farms. According to PGI  
104 regulations, calves remain with the suckler cows till weaned, often at pasture from spring to fall,  
105 and are fattened with traditional feeding practices based on forages and concentrates (compound  
106 feed and/or cereal mix). Silages are prohibited during the last two months of fattening. Slaughter  
107 date is decided by the farmer for each animal individually according to local market requirements,  
108 i.e. at carcass fatness scores of about 2.0 for young bulls and 2.5 for young heifers [14] on the  
109 European SEUROP carcass classification system [15].

110 All the animals sampled (83 young bulls, 8 heifers; 39 Chianina, 52 Romagnola) were  
111 registered in the Herd Books of their respective breeds, which are managed by the Associazione  
112 Nazionale Allevatori di Bovini Italiani da Carne (ANABIC; National Association of Italian Beef  
113 Cattle Farmers, Perugia, Italy). They were sired by 11 Chianina and 35 Romagnola bulls (mainly

114 through artificial insemination), representative of the current genetics of these beef breeds. The  
115 animals' ages, carcass weights, and daily carcass weight gains are presented in Table 1.

## 116 2.2. Meat samples

117 Animals were slaughtered in accordance with European Union regulations [16], and the  
118 carcass weight recorded. The day after slaughter, sample beef joints were obtained from the  
119 *Longissimus thoracis* muscle at the level of the division of the carcass sides into two quarters  
120 according the pistol cut (5<sup>th</sup> rib). Both sides of each carcass (182 meat samples in total) were  
121 sampled in order to assess the effects on the quality of meat sampled from the same anatomical  
122 position on the same animal. Samples were cooled, vacuum packed, and labeled, then taken to the  
123 DAFNAE Meat Laboratory, University of Padova, Italy, for analysis.

## 124 2.3. Analysis

### 125 2.3.1. Meat quality analysis

126 After 7 days of aging at 4 °C, the pH of the *Longissimus thoracis* samples was measured at  
127 three different points with a Delta Ohm HI-8314 pH meter. The average value of the three replicates  
128 was used for the subsequent analyses. The sample meat joints were then cut perpendicularly to the  
129 muscle fibers, and one hour later the color of the muscle surface was measured at five different  
130 points using a Minolta CM-508c (illuminate: D65, observer: 100) according to the procedure  
131 described by [17,18]. Mean color was expressed in L\*, a\*, b\*, C\* and h\* values [19].

132 To measure cooking losses, a 2cm-thick meat steak was placed in a polyethylene bag and cooked in  
133 a water bath at 70 °C for 40 minutes. Cooking loss was calculated as the percentage difference  
134 between the weight of the meat before and after cooking. The texture of the cooked meat sample  
135 was measured by shear force using a TA-HDi Texture Analyser (Stable Micro Systems, Godalming,  
136 United Kingdom) with a Warner Bratzler shear attachment (10N load cell, crosshead speed of 2  
137 mm/s) and analyzed with the Texture Expert software [20]. The average of three replicates was  
138 used for the meat texture analysis.

139 Chemical analysis of the meat was carried out according to [21]. Ash content was measured  
140 after drying the meat at 525 °C, according to the AOAC method, and lipid percentage was  
141 determined by the extraction method using petroleum ether. Cholesterol content was measured by  
142 extracting it by saponification and following the method described by [17].

### 143 2.3.2. Mineral analysis

144 The procedure used for analyzing the mineral content of the beef samples was reported in  
145 detail in a previous paper [22]. Briefly, after 7 days of aging at 4°C, fat from the outer side of each  
146 meat sample was inspected and trimmed. A sub sample of the meat was ground then freeze-dried.

147 The meat samples were analyzed for their mineral contents, and after identification the  
148 various minerals were quantified with a Spectro Arcos EOP ICP-OES (Spectro A.I. GmbH, Kleve,  
149 Germany). All instrument operating parameters were optimized for nitric acid 30% solution. The  
150 samples were analyzed after closed-vessel microwave digestion (Ethos 1600, Milestone S.r.l.,  
151 Sorisole, BG, Italy). Between 0.300 and 0.350 g of freeze-dried tissue from each sample was placed  
152 in a TFM vessel with 2 mL of 30% hydrogen peroxide and 7 mL of concentrated (65%) nitric acid,  
153 both Suprapur® quality (Merck Chemicals GmbH, Darmstadt, Germany). These prepared samples  
154 were subjected to microwave digestion as follows: Step 1, 25–200 °C in 15 min at 1200 W with P  
155 max 100 bar; Step 2, 200 °C for 15 min at 1200 W with P max 100 bar; Step 3, 200–110 °C in 15  
156 min. After cooling down to room temperature, the dissolved sample was diluted with ultrapure  
157 water (resistivity 18.2 M Ω cm at 25 °C) to a final volume of 25 mL.

158 Calibration standards were prepared using multi element and single element standard  
159 solutions (Inorganic Ventures Inc., Christiansburg, VA, USA) in 18% Suprapur® nitric acid to  
160 obtain similar matrices to the samples. Concentrations of 0, 0.005, 0.02, 0.05, 0.2, 0.5, 2 and 5  
161 mg/L of the analytes were prepared. The calibration solutions for calcium, potassium, magnesium,  
162 sodium, phosphorous and sulfur were at the same concentrations as the other analytes plus further  
163 concentrations of 20, 50 and 200 mg/L.

164 Method precision and trueness were assessed with a blank solution, a low-level control  
165 solution (recovery limits  $\pm 30\%$ ), a medium-level control solution (recovery limits  $\pm 10\%$ ), and the  
166 international standard reference material NIST SRM 1577c [National Institute of Standards &  
167 Technology (NIST), Gaithersburg, MD, USA], prepared as described above.

## 168 2.4. Statistical analysis

### 169 2.4.1 Mixed model analysis of variance

170 All data were first tested for normality and homogeneity. A comprehensive statistical  
171 analysis of variance of the data on animal performance and meat quality traits was then carried out  
172 using the PROC MIXED procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) to  
173 quantify the fixed (breed, sex) and random (farm/date, animal within farm/date, sample/side within  
174 animal) sources of variation of the traits. The following model was used for the analysis of variance:

$$175 y_{ijkl} = \mu + \text{breed}_i + \text{farm/date}(\text{breed})_{i;j} + \text{sex}_k + \text{animal}(\text{sex})_{k;l} + e_{ijkl},$$

176 where  $y_{ijkl}$  is the trait studied (animal performance and meat quality traits);  $\mu$  is the overall mean;  
177  $\text{breed}_i$  is fixed effect of breed ( $i = \text{Chianina, Romagnola}$ );  $\text{farm/date}(\text{breed})_{i;j}$  is the random effect of  
178 the  $j^{\text{th}}$  farm/slaughter date within breed ( $j = 1, \dots, 15$ ), which was used to test the significance of  
179 the breed effect;  $\text{sex}_k$  is the fixed effect of sex ( $k = \text{male, female}$ );  $\text{animal}(\text{sex})_{k;l}$  is the random effect  
180 of the  $l^{\text{th}}$  animal within sex ( $l = 1, \dots, 91$ );  $e_{ijkl}$  is the residual random error term referring to the  
181 differences between the meat samples taken from the two sides of each carcass,  $\sim N(0, \sigma^2)$ , where  $\sigma^2$   
182 is the side/residual variance. As age at slaughter, carcass weight and carcass daily gain had only one  
183 value per animal, they were analyzed with a reduced model, similar to the previous one but  
184 excluding the  $\text{animal}(\text{sex})$  effect, so that the residual term also summarizes the animal effect.

185 Tests for outliers were based on the residuals of the model for all the animal performance  
186 and meat quality traits before the final analysis of variance. Data which were within the range of  $\pm 3$   
187 residual standard deviations were kept, while the rest were treated as outliers and therefore omitted.

### 188 2.4.2 Correlations and multivariate statistical analysis



189 Farm/date and animal within farm/date correlations were analyzed to identify the  
190 relationships of the mineral contents to the meat quality traits and the animals' phenotypic traits,  
191 which yielded 640 correlation coefficients: 20 minerals  $\times$  16 animal traits (3 performance traits, 5  
192 meat composition traits, 3 meat physical quality traits, and 5 color traits)  $\times$  2 types of correlations  
193 (correlations among herd/date solutions and among animals within herd/date).

194 A multivariate analysis to capture the major part of the co-variation among the minerals was  
195 carried out and reported in a previous study (see Patel et al., 2019 [22] for details). Factor analysis  
196 was carried out in 3 steps using Varimax rotation in the R studio environment version 3.4.1 using  
197 the psych package. The eigenvalues of the factors and the communality values for the measured  
198 variables after rotation were also obtained. Five unmeasured latent explanatory independent factors  
199 were identified, explaining 69% of the total co-variation among the 20 minerals. Firstly, KMO  
200 (Kaiser-Meyer-Olkin) and Bartlett's test were carried out and confirmed that the mineral data were  
201 suitable for factor analysis.

202 The 5 unmeasured latent explanatory independent factors were:

- 203 1. Factor "*Quantity*": Eigen value 4.9, representing 45.2% of the co-variation explained by all  
204 factors, related to the meat content of P (loading 0.96), S (0.74), Mg (0.68), Cr (0.68), Al  
205 (0.64), Ti (0.71), Pb (0.78), Ba (0.53), and Sn (-0.74);
- 206 2. Factor "*Na+Fe+Cu*": Eigen value 2.2, representing 17.9% of the co-variation explained by  
207 all factors, related to the meat content of Na (loading 0.66), Fe (0.77), and Cu (0.60);
- 208 3. Factor "*K-B-Pb*": Eigen value 1.7, representing 15.6% of the co-variation explained by all  
209 factors, related to the meat content of K (loading 0.76), B (-0.53), and Pb (-0.54);
- 210 4. Factor "*Fe+Mn*": Eigen value 1.2, representing 10.8% of the co-variation explained by all  
211 factors, related to the meat content of Fe (loading 0.62), and Mn (0.48);
- 212 5. Factor "*Zn*": Eigen value 1.1, representing 10.4% of the co-variation explained by all factors,  
213 related to the meat content of Zn (loading 0.94).

214 The scores of each latent factor associated with the minerals in each meat sample were used to  
215 calculate the coefficients of the correlations of farm/date and animal within farm/date with animal  
216 performance and meat quality traits.

217

### 218 **3. Results**

#### 219 *3.1 Animal performance and meat quality and their sources of variation*

220 Descriptive statistics of animal performance and meat quality traits, and the significance levels of  
221 the fixed effects included in the model are presented in Table 1. The results show that the only  
222 differences due to breed that reached significance were in cooking losses, the shear force of cooked  
223 meat, and the lightness of raw meat. The Chianina beef was lighter than the Romagnola beef ( $L^*$   
224 33.5 vs. 36.8), but showed greater cooking losses (35.9 vs. 32.5%) and shear force (31.9 vs. 28.6  
225 N/cm<sup>2</sup>).

226 There were no differences in the meat chemical compositions of the two beef breeds,  
227 whereas the meat from the females had, as expected, greater dry matter and lipid contents than the  
228 meat from the males (27.7 vs. 25.9%, and 3.93 vs. 2.20%, respectively).

229 Analysis of the sources of variation treated as random factors, Figure 1 depicts the relative  
230 importance of group (farm/date of slaughter) and individual animal (carcass) within group to the  
231 total variance in beef performance traits, and of meat sample/side within animal (carcass) to the  
232 total variance in meat quality traits. It shows that the variability in all performance traits (age at  
233 slaughter, carcass weight and daily gain) is more affected by differences among groups (about two  
234 thirds of total variance) than by differences among animals within group (one third).

235 Meat quality traits, such as dry matter, lipids, color traits (excluding  $L^*$ ), and cooking losses  
236 were highly affected by farm/date of slaughter (around 50% of total variance). The effect of  
237 farm/date on ash, protein, cholesterol content and meat lightness was between 20 and 30% of total  
238 variance, whereas the effect on shear force and pH was lower than 10%.

239 The variability explained by individual animals was substantial for the meat content of lipids (> 50%  
240 of total variance), much lower (< 25%) for cholesterol and ash, and intermediate for all other meat  
241 quality traits. Lastly, the variability in ash and cholesterol content, shear force, and, in part, meat pH  
242 explained by sample/side within animal and the residual error was very high (> 50%). This means  
243 that the reproducibility of these traits is lower than 50%. The highest levels of reproducibility (>  
244 90%) were for the lipid and dry matter contents of meat and for the H\* color index, while the other  
245 meat quality traits had intermediate levels of reproducibility (65 to 85%).

246

### 247 *3.2 Mineral contents of meat and their latent explanatory independent factors*

248 A total of 20 minerals (Table 2), comprising 6 essential macro-minerals (Na, Mg, P, S, K,  
249 and Ca), 5 essential micro-minerals (Cr, Mn, Fe, Mn, and Zn) and 9 environmental micro-minerals  
250 (Li, B, Al, Ni, Sr, Sn, Ba, Ti, and Pb) were present in the meat samples in quantities above our limit  
251 of quantification (LOQ). A further 10 minerals (As, Be, Cd, Co, Hg, Mo, Sb, Se, Ti, and V) were  
252 also identified, but these were not present in at or above the LOQ in all the meat samples. The  
253 halogens (Cl, I, Br) were not identified as these minerals need special sample preparation and are  
254 detected at very low wavelengths, which require the instrument parameters to be reset with  
255 subsequent loss of precision. It is of note that the coefficients of variation in meat were very  
256 different for different minerals. In the case of the essential macro-minerals it was modest, ranging  
257 from 3% for K to 11% for Na, with the exception of Ca (28%). The coefficients of variation of the  
258 essential micro-minerals were in the range 13-17%, with the exception of Cr (51%), whereas those  
259 of the environmental micro-minerals were much larger, 35 to 64%, with the exception of Pb (22%).

260 A factor analysis of the mineral data matrix (91 animals x 20 minerals as variables) was  
261 carried out to condense all the relationships among the 20 minerals analyzed (190 correlation  
262 coefficients) in order to identify some common drivers among them that would facilitate  
263 interpretation of the results. Five latent factors were extracted.

264

### 265 3.3. Correlations between the detailed mineral profile of meat and animal performance

266 Table 3 summarizes the correlations between the animals' age at slaughter, carcass weight,  
267 and carcass gain on one side, and the detailed mineral profiles of the meat samples and their latent  
268 factors on the other. Two types of correlation were calculated: those among the effects of farm/date  
269 (groups of animals reared on the same farm and slaughtered on the same date) and those among the  
270 individual animal within farm/date groups. With few exceptions, the farm/date and animal  
271 correlations had the same sign and similar magnitudes, although the farm/date correlations were  
272 less often significant because of their fewer degrees of freedom. Given the high numbers of the  
273 minerals and the productive and qualitative traits that were correlated, when farm/date and animal  
274 correlations have the same sign and similar magnitudes, even though only one may be statistically  
275 significant, in reporting the results of this survey we will simply say that the two traits are  
276 correlated, without further specification.

277 Age at slaughter presented modest correlations with the meat mineral profile, although the positive  
278 correlations with Na, Mg, P, Al, Ti, and Ba, and the negative correlations with K, Fe, Zn, and Sn  
279 reached the threshold of statistical significance (Table 3).

280 Carcass weight and carcass daily gain exhibited fewer positive correlations with the mineral  
281 contents of beef, but those with Zn and Fe (and partly with S) were particularly strong. This  
282 complex situation is more clearly summarized by the latent factors: the factor *Quantity*, the most  
283 important, was correlated only with age at slaughter (positively, i.e., unfavorably); the factor  
284 *Na+Fe+Cu* was positively correlated with carcass weight and carcass gain; the factor *K-B-Pb* was  
285 not correlated at all with any of the performance traits; the factor *Fe+Mn* was correlated negatively  
286 with age at slaughter and positively with carcass weight; lastly, factor *Zn* was highly and positively  
287 correlated with both carcass weight and carcass gain.

288

### 289 3.4. Correlations between the detailed mineral profile and the chemical composition of meat

290 The farm/date and animal within farm/date correlations between the various minerals and  
291 the chemical composition of the meat that we analyzed are even more complex than those regarding  
292 animal performance traits. They are presented in Table 4.

293 Among the essential macro-minerals, only Na and P did not correlate with any of the meat chemical  
294 traits. Comparison of the meat samples from different animals within farm showed that Mg content  
295 was only modestly and negatively correlated with lipid content. Sulfur was positively correlated  
296 with the protein (and DM) content of meat, significantly when different animals were compared,  
297 non-significantly when different farm/dates were compared. Potassium was highly correlated with  
298 all chemical components (DM, protein, ash, cholesterol) except lipids. Calcium content had a strong  
299 positive farm/date correlation with the cholesterol content of meat, and modest positive animal  
300 correlations with protein, ash, and cholesterol content.

301 Among the essential micro-minerals, the only significant (and strong) farm/date correlations were  
302 those between Fe and Zn and the meat protein content (similar to the previously analyzed  
303 correlations with carcass weight and carcass gain). At the animal level, in addition to protein these  
304 metals were also correlated with DM, and only Zn with lipids. Mn was also correlated with DM,  
305 protein and lipids, whereas Cr was correlated (modestly) only with DM, and Cu was not correlated  
306 with any meat composition traits.

307 It is worth noting that several environmental micro-minerals were also related to meat  
308 composition traits. As expected for environmental minerals, some of them presented more  
309 significant farm/date correlations with meat composition (Table 4) than the other minerals. With a  
310 few exceptions, the animal correlations were of the same sign and similar magnitudes as the  
311 farm/date correlations, although they did not always reach the same level of statistical significance.  
312 Lithium was correlated negatively with lipid content and positively with ash and cholesterol  
313 contents. Boron and Pb were correlated negatively with all meat constituents, except lipids in the  
314 case of Pb, and, in the case of B, cholesterol, which, in contrast, was positively correlated with Al  
315 and Ba. Strontium was positively correlated with both cholesterol and ash. Nickel differed

316 somewhat in being correlated negatively with the DM and lipid content of meat at the farm/date  
317 level, but positively with DM at the animal level. The fact that environmental minerals have not so  
318 far been shown to play a biological role in farm animals does not mean that they are not absorbed  
319 and stored in some tissues and that these biological processes are not regulated by genetic,  
320 nutritional and health factors. The implications of the associations between some of these minerals  
321 and meat composition found here are largely unknown as there is almost nothing in the literature in  
322 this regard.

323 The latent explanatory factors of the meat mineral contents help simplify the analysis. The  
324 first two latent factors, which make the biggest contribution to explaining the overall mineral co-  
325 variance, showed no significant correlations with meat chemical composition. In contrast, the factor  
326 *K-B-Pb* was correlated with all chemical traits (dry matter, protein, ash, and cholesterol) except  
327 meat lipids (Table 6), the factor *Fe+Mn* with only meat protein content, and the factor *Zn* with dry  
328 matter, protein and lipids, and negatively with the ash content of meat.

329

### 330 *3.5. Correlations between the detailed mineral profile and the pH and physical properties of meat*

331 The farm/date of slaughter and the animal within farm/date correlations between the detailed  
332 mineral profile and the color traits of raw meat samples are summarized in Table 5, those with pH,  
333 cooking losses and shear-force of the cooked meat samples in Table 6.

334 Among the meat color traits, we found that lightness ( $L^*$ ) was not much affected by the mineral  
335 content, with the exception of the expected negative correlation with Fe, and the positive  
336 correlations with Ti and Pb.

337 Moving on to the color indices, all the macro-minerals in the meat, except K and Ca, were  
338 positively correlated with  $a^*$  (redness index) and  $b^*$  (yellowness index), and also, as expected from  
339 the calculations, with  $C^*$  (chroma) and (negatively) with  $H^*$  (hue). All the essential micro-minerals,  
340 except Cr, were also positively correlated with  $a^*$  (but not  $b^*$ ) and with  $C^*$ , and negatively with  $H^*$ .  
341 The associations between the environmental micro-minerals and meat color traits were more erratic:

342 Al and Ti were favorably associated with a\*, b\*, and C\*; Li and Sn were negatively associated with  
343 the same traits; Sr was positively associated only at the farm/date level and only with H\*; while the  
344 B, Ni, and Ba variations were independent of meat color traits.

345 Here, too, the latent explanatory factors helped simplify the picture: the factor *Quantity* was  
346 correlated with all the color traits (negatively in the case of H\*); the factor *Na+Fe+Cu* was only  
347 modestly correlated with a\* and H\*; the factor *K-B-Pb* was negatively correlated with lightness, but  
348 was not correlated with meat color indices; the factor *Fe+Mn* was, as expected, correlated  
349 negatively with meat lightness, positively with a\* and negatively with H\*; lastly, the factor Zn was  
350 correlated positively with a\* and C\*, and negatively with H\*.

351 The relationships between the mineral content and the other meat quality traits are simpler. The  
352 acidity of meat (pH) was associated at the farm/date level only with Sr content. Tenderness (shear  
353 force) was not associated with any of the minerals in the meat. Cooking losses presented some  
354 significant correlations: negatively (favorably) with some essential minerals (S, K and Zn), and  
355 positively with two environmental minerals (B and Pb). In this case, the latent explanatory factors  
356 do not reveal many relationships, the only significant one being the correlation between factor *K-B-*  
357 *Pb* and meat cooking losses (Table 6).

358

## 359 **4. Discussion**

### 360 *4.1 Animal performance and meat quality traits*

361 Animal performance and meat quality *per se* were not a primary objective of this study, but  
362 we needed a preliminary analysis of them to understand and interpret the complex relationships  
363 with the mineral profile of meat.

364 Results show that the differences due to the animal's breed or sex relative to age at slaughter,  
365 carcass weight, and carcass gain did not reach significance. It is worth mentioning that the beef  
366 breeds of Central Italy have a common ancestry [23], and their rearing in accordance with European  
367 Union PGI specifications for "Vitellone bianco dell'Appennino Centrale" is a traditional operation



368 aimed at high quality production. The majority of the young bulls and heifers are reared on small-  
369 medium farms, and both the cow-calf and fattening phases have one of the highest gross margins  
370 per cow in the EU [24]. The performance traits measured in this study are very similar to those  
371 reported in a previous large survey of more than 20,000 animals [14], which also found that  
372 Chianina cattle have a greater carcass weight than the Romagnola (430 kg vs. 367 kg, respectively),  
373 as well as a greater carcass gain (0.64 kg/day vs. 0.54 kg/day) at similar ages at slaughter.

374 The results for the chemical and physical traits are in the range of those reported by [25–27],  
375 except for the b\*, C\* and h\* color parameters, which are little higher. This could be due to the fact  
376 the animals sampled for those studies came from single farms.

377 We have clearly shown that the effect of animal group, i.e. animals from the same farm  
378 slaughtered on the same day (farm/date effect), was the most important for almost all animal  
379 performance and meat quality traits (Figure 1), accounting for between one to two thirds of their  
380 total variance. The only exceptions were for pH, ash and cholesterol contents, and meat shear force  
381 (5 to 25% of total variance represented by farm/date). It was not the objective of this survey to  
382 analyze in detail the effects of different management and feeding practices on the PGI farms, which  
383 would have required us to sample a much larger number of farms, but rather to obtain an overview  
384 of the average productive and qualitative traits, and of their variability and relationships with the  
385 detailed mineral profile of meat. In a very large survey carried out on another Italian beef breed  
386 (Piemontese) reared in north-west Italy in accordance with another set of PGI regulations [28], the  
387 authors were able to disentangle the effect of farm from that of date of slaughter, and found that the  
388 latter was often more important than the former. They also noted that the variation between  
389 individual farms within a common beef farming system is often more important than the variation  
390 between different farming systems [29]. Our results clearly show that a first level of analysis of the  
391 relationships between meat quality and the mineral profile should be the farm/date level.

392 Our results also show that the variation among individual animals within farm/date group is only  
393 slightly less important than the variation between different groups of animals (Figure 1), confirming



394 the results of [28]. This reveals the need for a second level of analysis focusing on individual  
395 animals/carcasses. The third source of variation (among different samples within animal/carcass)  
396 was generally modest, with the exception of a few traits characterized by low reproducibility (pH,  
397 ash, and cholesterol contents, and meat shear force).

398

#### 399 *4.2 Mineral profile of beef and its relationship with animal performance and meat quality*

400 Similarly to what we found for meat quality traits, the mineral profile was hardly affected by  
401 the animal's breed and sex, as the only significant differences were among breeds for the content of  
402 Ca and B, and between young bulls and heifers for the content of K, Zn, Sn, and Pb [22]. Very few  
403 of the many studies carried out on the mineral content of beef have compared different cattle breeds  
404 [30–33] or sexes [34–36], and most have confirmed the modest effects of these sources of variation.  
405 From these results, it seems unnecessary to study the relationships between the mineral profile and  
406 meat quality within specific breeds or sexes.

407 As we observed for animal performance and meat quality traits, in the case of the mineral  
408 content of beef we also found considerable variability in the relative importance of farm/date,  
409 animal/carcass within farm/date, and meat sample within animal/carcass. In particular, farm/date  
410 was the most important source of variation for Na, Mg, P, S, Li, Al, Sn, and Ti; individual  
411 animal/carcass was the most important for Mn, B, and Sr; these two sources were equally important  
412 for Fe, Cu, Zn, and Pb; and, lastly, meat sample within animal/carcass was the most important for K,  
413 Ca, Cr, Ni, and Ba [22].

414 The sources of variation in the meat mineral profile, like those in animal performance and  
415 meat quality, also confirm the need for at least two levels of analysis with respect to the co-  
416 variation between these two groups of traits: the farm/date level and the animal/carcass within  
417 farm/date level.

418 The results summarized in Tables 3, 4, 5, and 6 show the 320 correlations (52 of them  
419 significant) between the 20 minerals and the 16 animal performance and meat quality traits at the

420 level of farm/date effects, and another 320 correlations (101 significant) at the level of individual  
421 animal/carcass within farm/date.

422 The scientific literature contains some studies that also report obtaining correlation  
423 coefficients [37], often as Pearson correlations, among the raw data without making any distinction  
424 between different levels of analysis. While our data are too numerous to facilitate understanding of  
425 the interrelationships between mineral contents and meat quality, the few data that can be found in  
426 the literature are too heterogeneous and erratic to allow the same objective to be reached.

427

#### 428 *4.3 Latent factors of the beef mineral profile and their relationships with animal performance and* 429 *meat quality*

430 The fact that the content of one mineral in meat is not independent of the content of another,  
431 and that there are certain common genetic and physiological mechanisms of absorption, storage,  
432 mobilization and excretion, complicate the analysis. In the previous study [22], we examined the  
433 190 correlations among these 20 minerals at the farm/date level, and the 190 correlations at the  
434 animal/carcass within farm/date level, and obtained numerous high correlation coefficients within  
435 each group, both positive and negative. The farm/date correlations reflect the effect of different  
436 environmental conditions, facilities, management systems, and feeding strategies, whereas the  
437 animal correlations reflect the variability among animals in the same external conditions due to  
438 their genetics, physiology, and health.

439 We therefore carried out a multivariate analysis of the mineral dataset, which yielded 5 unmeasured  
440 latent explanatory factors, fully independent of each other, that summarized 69% of the co-variation  
441 in the minerals [22]. Multivariate analyses have been used in previous studies, especially for  
442 authenticating meat origin or production systems [38, 39], but we have found no other studies using  
443 a factor analysis of the mineral content of beef to investigate correlations with meat quality.

444 The most important latent factor, “*Quantity*”, which related to the concentrations of almost  
445 half the minerals analyzed (9 out of 20), all with positive loadings except Sn, and explained 45% of

446 their total co-variance, did not correlate well with the traits studied. Among the animal performance  
447 traits, it was positively correlated only with age at slaughter at the level of individual animals within  
448 farm/date groups. It is worth pointing out that age at slaughter also has a genetic aspect as it reflects  
449 the earliness or lateness of maturation of the animals [14], and is negatively correlated with carcass  
450 weight, carcass gain and fat deposition, which could reflect greater mineral deposition in the meat  
451 of animals that require a longer fattening period to reach the level of maturation required by the  
452 market.

453 As we have seen, the only meat characteristic affected by the latent factor “*Quantity*” was  
454 beef color. The meat samples with the highest scores for this latent factor were often also those with  
455 a greater intensity of color ( $a^*$ ,  $b^*$ , and  $C^*$ ), and greater lightness (Table 5). This cannot be  
456 attributed to the effect of myoglobin because Fe is not among the minerals characterizing this latent  
457 factor (its loading is -0.03).

458 Fe is one of the minerals characterizing the second latent factor, “*Na+Fe+Cu*”, which  
459 explains almost 18% of the total factor variation, and also the fourth latent factor, “*Fe+Mn*”, which  
460 explains almost 11% of factor variation. As expected, both were positively correlated with the  
461 redness index ( $a^*$ ), and negatively with the Hue index ( $H^*$ ) (Table 5), while the factor “*Fe+Mn*”  
462 was also negatively correlated with meat lightness ( $L^*$ ). All these effects are, of course, explained  
463 by the role Fe plays in the oxidative metabolism of the muscle [40], and particularly the constituent  
464 role it plays in myoglobin, hemoglobin, and cytochromes, which could also explain the positive  
465 association of “*Na+Fe+Cu*” with both carcass weight and carcass gain (Table 3). A combined  
466 deficiency of Fe and Cu in the diet is known to reduce the growth rate of young cattle [1], although  
467 this type of deficiency is not common. Excessive Fe intake, however, could result in the depletion  
468 of Cu in cattle and hence increase the dietary requirement of this mineral [41,42]. All these  
469 relationships were significant when individual animals within groups were compared, but not when  
470 different farm/date groups were compared. At this second level, we only found a high positive  
471 correlation (+0.64) between “*Fe+Mn*” and the crude protein content of meat (Table 4). Fe and Mn

472 are known to interact: high levels of Fe have been shown to reduce the activity of the transporters  
473 involved in the metabolism of Fe and Mn, and to increase intestinal permeability in calves [43].

474 The third latent factor, “*K-B-Pb*”, explaining almost 16% of factor variation, was the most  
475 correlated with meat chemical composition (Table 4). It is worth noting that K was negatively  
476 correlated with B and Pb at both the farm/date and animal/carcass within farm/date levels [22],  
477 which explains why it has a positive loading (+0.76) in this factor, whereas B and Pb have negative  
478 loadings (-0.53 and -0.54, respectively). Moreover, K is the individual mineral most positively  
479 correlated with the chemical composition of meat, and B and Pb are the individual minerals most  
480 negatively correlated with the composition of meat (lipids excluded). The role of B and Pb in meat  
481 is not known, but the highest correlations of “*K-B-Pb*” are with meat ash content, which could be  
482 explained by the fact that K is the most abundant mineral in meat (Table 2). Moreover, K in meat is  
483 involved in muscle contraction and nerve impulses, and some enzymatic reactions [1]. The animal’s  
484 body has a very limited capacity to store K, so deficiency can develop rapidly, causing, among  
485 other things, muscle weakness [44].

486 The latent factor “*K-B-Pb*” also correlated negatively with L\* (Table 5), and, in particular,  
487 with cooking losses (Table 6). The fact that K is the major cation in intracellular fluids, hence its  
488 importance in acid-base balance, osmotic pressure and water balance [1], could be the reason why it  
489 correlates with the loss of liquids during cooking.

490 Lastly, the fifth latent factor, the only one associated with just one mineral, “*Zn*”, explaining  
491 just over 10% of total factor variance, also correlated positively with dry matter, protein and lipid  
492 content, and negatively with the ash content of meat (Table 4), but it also seemed to be related to  
493 beef color (correlating positively with a\* and C\*, negatively with H\*, Table 5). The highest  
494 correlations showed by this latent factor were not with meat quality but with animal performance  
495 traits, particularly carcass weight and carcass gain, at the farm/date and animal/carcass levels (Table  
496 3). These many and important correlations are testimony to the importance of Zn in several aspects  
497 of the animal’s metabolism: it is an essential component of many important metalloenzymes and

498 also triggers other enzymatic activities that affect the metabolism of carbohydrates, proteins, lipids  
499 and nucleic acids [45]. Zn deficiency, in addition to its well-known effects on the tegumental  
500 apparatus (swollen feet, parakeratotic lesions of the skin), also impairs growth, feed intake and feed  
501 efficiency [1].

502

## 503 **5. Conclusions**

504 This study had clearly shown that the animal's breed and sex have limited effects on the  
505 meat mineral profile, whereas variability in groups (animals from the same farm and same date of  
506 slaughter), individual animal within group, and individual sample within animal differed greatly  
507 according to mineral and according to quality trait. Moreover, the minerals in meat correlated with  
508 animal performance and meat quality traits in very complex ways. A multivariate analysis of the  
509 mineral dataset resulted in 69% of the co-variation in minerals being condensed into 5 unmeasured  
510 latent explanatory factors characterized by co-variation in 1 to 9 minerals. Two latent factors related  
511 to Fe content (“*Na+Fe+Cu*” and “*Fe+Mn*”) were found to play a special role in meat color traits, a  
512 further two (“*K-B-Pb*” and “*Zn*”) played a special role in meat chemical composition, and only “*Zn*”  
513 in carcass weight and daily gain, and meat color. Meat cooking losses were affected by “*K-B-Pb*”,  
514 whereas meat shear force was not related to any latent factor nor any individual mineral in the meat.  
515 These latent factors simplify the picture of the relationships between the minerals and animal  
516 performance and meat quality traits, facilitating the comprehension and interpretation of the role  
517 minerals.

518

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531 **References**

- 532 1. National Academies of Sciences, Engineering, and Medicine. Nutrient Requirements of Beef  
533 Cattle: Eighth Revised Ed.; The National Academies Press: Washington, DC, USA, 2016; pp.  
534 4-138.
- 535 2. McAfee, A.J.; McSorley, E.M.; Cuskelly, G.J.; Moss, B.W.; Wallace, J.M.W.; Bonham,  
536 M.P.; Fearon, A.M. Red meat consumption: An overview of the risks and benefits. *Meat Sci.*  
537 **2010**, *84*, 1-13.
- 538 3. Rooke, J.A.; Flockhart, J.F.; Sparks, N.H. The potential for increasing the concentrations of  
539 micro-nutrients relevant to human nutrition in meat, milk and eggs. *J. Agric. Sci.* **2010**, *148*,  
540 603-614.
- 541 4. Greenfield, H.; Southgate, D.A.T. Food composition data. Production, management and use.  
542 *Food Agric. Organ. United Nations* **1996**, *57*, 1-47.
- 543 5. Ramos, A.; Cabrera, M.C.; Saadoun, A. Bioaccessibility of Se, Cu, Zn, Mn and Fe, and heme  
544 iron content in unaged and aged meat of Hereford and Braford steers fed pasture. *Meat Sci.*  
545 **2012**, *91*, 116-124.
- 546 6. Schönfeldt, H.C.; Hall, N. Nutrient content of South African red meat and the effect of age  
547 and production system. *South African Journal of Animal Science.* **2015**, *45*, 313-324.
- 548 7. Domaradzki, P.; Florek, M.; Staszowska, A.; Litwińczuk, Z. Evaluation of the Mineral  
549 Concentration in Beef from Polish Native Cattle. *Biol. Trace Elem. Res.* **2016**, *171*, 328-332.
- 550 8. Czerwonka, M.; Szterk, A. The effect of meat cuts and thermal processing on selected  
551 mineral concentration in beef from Holstein-Friesian bulls. *Meat Sci.* **2015**, *105*, 75-80.
- 552 9. Jorhem, L.; Sundström, B.; Åstrand, C.; Haegglund, G. The levels of zinc, copper,  
553 manganese, selenium, chromium, nickel, cobalt, and aluminium in the meat, liver and kidney  
554 of swedish pigs and cattle. *Z. Lebensm. Unters. Forsch.* **1989**, *188*, 39-44.
- 555 10. Nutrient Data Laboratory USDA Food Composition Databases. *Natl. Nutr. Database Stand.*  
556 *Ref.* **2016**.
- 557 11. Giddings, G.G.; Solberg, M. *The Basis of Color in Muscle Foods*; 1977; Vol. 9; ISBN  
558 1040839770.
- 559 12. Shen, Q. W.; Min, D. Przybylski, W. Conversion of muscle to meat. *Meat Quality: Genetic*  
560 *and Environmental Factors*, 1<sup>st</sup> ed.; Wiesław, P., Hopkins, D; CRC press: Boca Raton, FL  
561 33487, USA 2016; pp. 81-100.
- 562 13. Kemp, C.M.; Sensky, P.L.; Bardsley, R.G.; Buttery, P.J.; Parr, T. Tenderness - An enzymatic  
563 view. *Meat Sci.* **2010**, *84*, 248-256.
- 564 14. Sbarra, F.; Mantovani, R.; Quaglia, A.; Bittante, G. Genetics of slaughter precocity, carcass  
565 weight, and carcass weight gain in Chianina, Marchigiana, and Romagnola young bulls  
566 under protected geographical indication. *J. Anim. Sci.* **2013**, *91*, 2596-2604.
- 567 15. Anonymous. Community scale for the classification of carcasses of adult bovine animals.  
568 Luxembourg: Off. Publ. Eur. Communities No. 1208/81, 2939/81 and 1026/91.16.  
569 Commission, T.H.E.; The, O.F.; Communities, E. 20.12.2006. 2006, *2006*, 5-24.



- 570 16. Council, E. Council regulation (EC) No 1099/2009. *Off. J. Eur. Union* 2009, 1-30.
- 571 17. Schiavon, S.; De Marchi, M.; Tagliapietra, F.; Bailoni, L.; Cecchinato, A.; Bittante, G. Effect  
572 of high or low protein ration combined or not with rumen protected conjugated linoleic acid  
573 (CLA) on meat CLA content and quality traits of double-muscled Piemontese bulls. *Meat Sci.*  
574 **2011**, *89*, 133-142.
- 575 18. Schiavon, S.; Tagliapietra, F.; Cesaro, G.; Gallo, L.; Cecchinato, A.; Bittante, G. Lowcrude  
576 protein diets and phase feeding for double-muscled crossbred young bulls and heifers.  
577 *Livestock Sci.* **2013**, *157*, 462-470.
- 578 19. CIE Recommendations on Uniform Color Spaces, Color-Difference Equations, and Metric  
579 Color Terms. *Color Res. Appl.* 1977, *2*, 5-6.
- 580 20. Joseph, R.L. The Future of Beef Production in the European Community. *Futur. Beef Prod.*  
581 *Eur. Community* **1979**, 596-600.
- 582 21. AOAC Official Method 942.05 - Ash. *AOAC Off. Methods Anal.* 2000, chapter 4, 5-15.
- 583 22. Patel, N.; Bergamaschi, M.; Cagnin, M.; Bittante, G. Sources of variation and latent  
584 explanatory factors of detailed mineral profile of beef. *Int. J. of Food Sci. & Tech.* **2019**  
585 (under review)
- 586 23. Maretto, F.; Ramljak, J.; Sbarra, F.; Penasa, M.; Mantovani, R.; Ivanković, A.; Bittante, G.  
587 Genetic relationships among Italian and Croatian Podolian cattle breeds assessed by  
588 microsatellite markers. *Livest. Sci.* **2012**, *150*, 256-264.
- 589 24. EC Farm Economics brief N°2 EU production costs overview. European Commission  
590 Agriculture and Rural Development, 2011, 1-14.
- 591 25. Preziuso, G.; Russo, C. Meat quality traits of *longissimus thoracis*, *semitendinosus* and  
592 *triceps brachii* muscles from Chianina beef cattle slaughtered at two different ages. *Ital. J.*  
593 *Anim. Sci.* **2004**, *3*, 267-273.
- 594 26. Mattii, S.; Priori, S.; Trombetta, M.F. Influence of sunflower cake supplementation on  
595 Marchigiana carcass and meat quality. *Ital. J. Anim. Sci.* **2009**, *8*, 513-515.
- 596 27. Marino, R.; Albenzio, M.; della Malva, A.; Caroprese, M.; Santillo, A.; Sevi, A. Changes in  
597 meat quality traits and sarcoplasmic proteins during aging in three different cattle breeds.  
598 *Meat Sci.* **2014**, *98*, 178-186.
- 599 28. Savoia, S.; Brugiapaglia, A.; Pauciullo, A.; Di Stasio, L.; Schiavon, S.; Bittante, G.; Albera,  
600 A. Characterisation of beef production systems and their effects on carcass and meat quality  
601 traits of Piemontese young bulls. *Meat Sci.* **2019a**, *153*, 75-85.
- 602 29. Savoia, S.; Albera, A.; Brugiapaglia, A.; Stasio, L. Di; Cecchinato, A.; Bittante, G.; Science,  
603 F. Prediction of meat quality traits in the abattoir using portable and hand-held near-infrared  
604 spectrometers : validation , repeatability and field testing. *Meat Sci.* **2019b** (under review)
- 605 30. Pilarczyk, R. Elemental composition of muscle tissue of various beef breeds reared under  
606 intensive production systems. *Int. J. Environ. Res.* **2014a**, *8*, 931-940.
- 607 31. Pilarczyk, R. Concentrations of toxic and nutritional essential elements in meat from  
608 different beef breeds reared under intensive production systems. *Biological Trace Element*  
609 *Research.* **2014b**, *158*, 36-44.



- 610 32. Litwińczuk, Z.; Domaradzki, P.; Florek, M.; Zólkiewski, P.; Staszowska, A. Content of  
611 macro- and microelements in the meat of young bulls of three native breeds (Polish Red,  
612 White-Backed and Polish Black-and-White) in comparison with Simmental and Polish  
613 Holstein-Friesian. *Ann. Anim. Sci.* **2015**, *15*, 977-985.
- 614 33. Pereira, V.; Carbajales, P.; López-Alonso, M.; Miranda, M. Trace Element Concentrations in  
615 Beef Cattle Related to the Breed Aptitude. *Biol. Trace Elem. Res.* **2018**, *186*, 135-142.
- 616 34. Doornenbal, H.; Murray, A.C. Effects of Age, Breed, Sex and Muscle on Certain Mineral  
617 Concentrations in Cattle. *J. Food Sci.* **1982**, *47*, 55-58.
- 618 35. Alonso, M.L.; Benedito, J.L.; Miranda, M.; Castillo, C.; Hernández, J.; Shore, R.F.  
619 Interactions between toxic and essential trace metals in cattle from a region with low levels  
620 of pollution. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 165-172.
- 621 36. Alonso, M.L.; Montaña, F.P.; Miranda, M.; Castillo, C.; Hernández, J.; Benedito, J.L.  
622 Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe,  
623 Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *BioMetals* **2004**, *17*,  
624 389-397.
- 625 37. Garmyn, A.J.; Hilton, G.G.; Mateescu, R.G.; Morgan, J.B.; Reecy, J.M.; Tait, J.G.; Beitz,  
626 D.C.; Duan, Q.; Schoonmaker, J.P.; Mayes, M.S.; et al. Estimation of relationships between  
627 mineral concentration and fatty acid composition of longissimus muscle and beef palatability  
628 traits. *J. Anim. Sci.* **2011**, *89*, 2849-2858.
- 629 38. Sun, S.; Guo, B.; Wei, Y.; Fan, M. Geographical origin traceability of lamb based on mineral  
630 element fingerprints. *Trans. of the Chinese Society of Agri. Engg.* **2012**, *28*, 237-243.
- 631 39. Zhao, Y.; Wang, D.; Yang, S. Effect of organic and conventional rearing system on the  
632 mineral content of pork. *Meat Sci.* **2016**, *118*, 103-107.
- 633 40. McDowell, L.R. Minerals in Animal and Human Nutrition, Second Ed.; Elsevier:  
634 Amsterdam, The Netherlands. 2003; pp. 203-233.
- 635 41. Bremner, I.; Humphries, W.R.; Phillipppo, M.; Walker, M.J.; Morrice, P.C. Iron-induced  
636 copper deficiency in calves: Dose-response relationships and interactions with molybdenum  
637 and sulphur. *Anim. Prod.* **1987**, *45*, 403-414.
- 638 42. Garthwaite, P.H.; Humphries, W.R. The effect of dietary molybdenum and iron on copper  
639 status and growth in cattle. *J. Agric. Sci.* **1987**, *109*, 315-320.
- 640 43. Hansen, S.L.; Ashwell, M.S.; Moeser, A.J.; Fry, R.S.; Knutson, M.D.; Spears, J.W. High  
641 dietary iron reduces transporters involved in iron and manganese metabolism and increases  
642 intestinal permeability in calves. *J. Dairy Sci.* **2010**, *93*, 656-665.
- 643 44. Devlin, T.J.; Roberts, W.K.; St Omer, V. V. Effects of dietary potassium upon growth, serum  
644 electrolytes and intrarumen environment of finishing beef steers. *J. Anim. Sci.* 1969, *28*, 557-  
645 562.
- 646 45. Hambidge, K.M.; Casey, C.E.; Krebs, N.F. Zinc. Trace Elements in Human and Animal  
647 Nutrition, Fifth Ed.; Mertz, W.; Academic Press: London, UK. 2012; Volume 2, pp. 1-137.

649 **Table 1.** Descriptive statistics of animal performance and meat composition, quality and color  
 650 traits and significance of fixed breed and sex factors analyzed using a mixed model.  
 651

	N <sup>1</sup>	Mean	SD	Min	Max	Breed	Sex	RMSE
<i>Animal performance:</i>								
Age at slaughter, d	91	701	34.0	589	731	-	-	21.17
Carcass weight, kg	91	403	90.7	196	572	-	-	45.74
Carcass gain, kg/d	91	0.58	0.14	0.27	0.90	-	-	0.07
<i>Meat composition:</i>								
DM, %	177	25.9	1.7	22.7	32.6	-	<i>P</i> <0.01	0.46
Ash, %	178	1.1	0.1	1.0	1.2	-	-	0.03
Protein, %	182	22.2	0.7	20.5	24.2	-	-	0.39
Lipid, %	173	2.3	1.2	0.4	8.3	-	<i>P</i> <0.01	0.21
Cholesterol, mg/100 g	180	54.2	4.3	43.4	64.3	-	-	3.41
<i>Meat quality traits:</i>								
pH	177	5.7	0.1	5.5	6.0	-	-	0.08
Cooking loss, %	179	34.6	2.8	25.9	40.0	<i>P</i> <0.01	-	1.13
Shear force, (N/cm <sup>2</sup> )	179	30.1	6.5	14.3	49.6	<i>P</i> <0.05	-	5.03
<i>Meat color traits:</i>								
L*	182	34.3	3.4	23.8	46.2	<i>P</i> <0.05	-	1.92
a*	182	14.3	3.2	6.3	21.2	-	-	1.38
b*	181	13.0	2.4	4.6	19.3	-	-	1.28
C*	181	19.3	3.8	8.1	28.3	-	-	1.79
H*	180	42.2	3.9	33.5	54.4	-	-	1.42

652 <sup>1</sup>The number of sides sampled is 182 (91 carcasses × 2 sides each) and number of data of meat quality traits lower than  
 653 182 reflect editing of data.

654 **Table 2.** Descriptive statistics (mean and standard deviation) of the essential and environmental  
 655 macro- and micro-minerals analyzed in meat samples.

656

Element	Minerals	Atomic number	Category	Mean <sup>1</sup>	SD <sup>1</sup>
<i>Macro-minerals:</i>					
Na	Sodium	11	Alkali metal	436	46
Mg	Magnesium	12	Alkaline earth metal	179	13
P	Phosphorus	15	Polyatomic nonmetal	1,488	144
S	Sulfur	16	Polyatomic nonmetal	1,354	78
K	Potassium	19	Alkali metal	2,821	79
Ca	Calcium	20	Alkaline earth metal	46	13
<i>Ess. micro minerals:</i>					
Cr	Chromium	24	Transition metal	12.5	6.4
Mn	Manganese	25	Transition metal	47.6	8.1
Fe	Iron	26	Transition metal	14,260	2,373
Cu	Copper	29	Transition metal	460	60
Zn	Zinc	30	Post-transition metal	39,782	5,395
<i>Env. micro-minerals:</i>					
Li	Lithium	3	Alkali metal	4.1	1.7
B	Boron	5	Metalloid	164	105
Al	Aluminium	13	Post-transition metal	755	263
Ti	Titanium	22	Transition metal	14	5.1
Ni	Nickel	28	Transition metal	20.6	11.6
Sr	Strontium	38	Alkaline earth metal	47.3	20.3
Sn	Tin	50	Post-transition metal	350	154
Ba	Barium	56	Alkaline earth metal	11.2	5.9

657 <sup>1</sup>: on fresh basis

658

**Table 3.** Herd ( $r_H$ ) and animal ( $r_A$ ) correlation coefficients of animal performance with latent explanatory factors and with individual essential and environmental macro- and micro-minerals concentration in meat

Elements	Age at slaughter, d		Carcass weight, kg		Carcass gain, kg/d	
	$r_H$	$r_A$	$r_H$	$r_A$	$r_H$	$r_A$
<i>Latent factors</i>						
F1 <i>Quantity</i>	0.25	<b>0.31**</b>	0.14	-0.04	0.05	0.05
F2 <i>Na+Fe+Cu</i>	-0.14	-0.13	0.35	<b>0.25*</b>	0.35	<b>0.24*</b>
F3 <i>K-B-Pb</i>	-0.17	-0.16	-0.20	-0.02	-0.12	-0.08
F4 <i>Fe+Mn</i>	-0.37	<b>-0.25*</b>	0.40	<b>0.23*</b>	0.44	0.19
F4 <i>Zn</i>	-0.32	-0.21	<b>0.78**</b>	<b>0.62***</b>	<b>0.74**</b>	<b>0.64***</b>
<i>Macro-minerals</i>						
Na	0.25	<b>0.24*</b>	0.08	-0.01	0.01	0.06
Mg	0.18	<b>0.28**</b>	0.09	-0.11	0.03	-0.04
P	0.22	<b>0.29**</b>	0.15	-0.03	0.06	0.06
S	0.02	0.05	0.50	<b>0.32**</b>	0.42	<b>0.39**</b>
K	-0.34	<b>-0.21*</b>	-0.07	0.10	0.03	0.05
Ca	0.35	0.19	-0.13	-0.09	-0.21	-0.04
<i>Ess. micro-minerals</i>						
Cr	0.17	0.13	0.04	0.02	-0.01	0.06
Mn	-0.22	-0.08	0.31	0.16	0.33	0.16
Fe	-0.40	<b>-0.29*</b>	<b>0.82***</b>	<b>0.60***</b>	<b>0.81***</b>	<b>0.59***</b>
Cu	0.37	0.19	0.21	0.01	0.09	0.05
Zn	-0.29	<b>-0.21*</b>	<b>0.79***</b>	<b>0.65***</b>	<b>0.75**</b>	<b>0.68***</b>
<i>Env. micro-minerals</i>						
Li	-0.33	-0.02	-0.29	<b>-0.26*</b>	-0.15	<b>-0.31**</b>
B	0.04	0.09	-0.01	-0.05	0.01	-0.04
Al	0.28	<b>0.22*</b>	-0.04	-0.12	-0.12	-0.06
Ti	0.21	<b>0.21*</b>	0.14	0.02	0.06	0.09
Ni	0.19	0.17	-0.37	0.04	-0.34	-0.01
Sr	0.28	0.17	<b>-0.61*</b>	<b>-0.34**</b>	<b>-0.62*</b>	<b>-0.32**</b>
Sn	-0.27	<b>-0.27**</b>	-0.06	0.08	0.03	0.01
Ba	0.52	<b>0.21**</b>	-0.29	-0.14	-0.38	-0.09
Pb	0.01	0.04	0.06	0.01	0.05	0.02

\* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$

**Table 4.** Farm/date ( $r_F$ ) and animal within farm/date ( $r_A$ ) correlation coefficients of chemical composition with latent explanatory factors and with individual essential and environmental macro- and micro-minerals concentration in meat.

Elements	DM		Protein		Lipid		Ash		Cholesterol	
	$r_F$	$r_A$	$r_F$	$r_A$	$r_F$	$r_A$	$r_F$	$r_A$	$r_F$	$r_A$
<i>Latent factors</i>										
F1 <i>Quantity</i>	-0.05	-0.09	-0.15	-0.05	-0.02	-0.11	-0.38	-0.16	0.17	0.03
F2 <i>Na+Fe+Cu</i>	-0.23	0.08	-0.01	0.12	-0.26	0.01	-0.11	0.15	0.14	0.05
F3 <i>K-B-Pb</i>	0.35	<b>0.34**</b>	<b>0.55*</b>	<b>0.62***</b>	0.29	0.16	<b>0.79**</b>	<b>0.71***</b>	<b>0.69**</b>	<b>0.34**</b>
F4 <i>Fe+Mn</i>	0.32	0.12	<b>0.64*</b>	0.19	0.12	0.04	0.17	0.08	0.10	0.08
F4 <i>Zn</i>	0.48	<b>0.40**</b>	<b>0.61*</b>	<b>0.29*</b>	0.37	<b>0.37**</b>	-0.20	<b>-0.24*</b>	-0.17	-0.07
<i>Macro-minerals</i>										
Na	-0.09	-0.07	-0.10	-0.13	-0.07	-0.04	-0.25	-0.09	0.30	0.12
Mg	-0.11	-0.13	-0.11	0.07	-0.09	<b>-0.21*</b>	-0.22	0.07	0.32	0.15
P	-0.07	-0.05	-0.13	0.03	-0.05	-0.11	-0.32	-0.01	0.23	0.11
S	0.24	<b>0.29**</b>	0.30	<b>0.49***</b>	0.18	0.09	-0.21	0.14	0.31	0.17
K	0.24	<b>0.37**</b>	0.51	<b>0.59***</b>	0.20	0.17	<b>0.69**</b>	<b>0.67***</b>	<b>0.74**</b>	<b>0.35**</b>
Ca	0.03	0.12	0.26	<b>0.23*</b>	-0.07	0.03	0.34	<b>0.24*</b>	<b>0.76**</b>	<b>0.24*</b>
<i>Ess. micro-minerals</i>										
Cr	0.32	<b>0.22*</b>	0.07	0.20	0.41	0.19	0.18	0.05	0.04	0.05
Mn	0.38	<b>0.27*</b>	0.39	<b>0.22*</b>	0.30	<b>0.21*</b>	0.001	-0.03	0.30	-0.02
Fe	0.21	<b>0.26*</b>	<b>0.53*</b>	<b>0.31**</b>	0.06	0.14	-0.26	-0.02	0.01	-0.02
Cu	-0.14	-0.16	-0.21	-0.07	-0.10	-0.16	-0.40	-0.07	0.16	0.01
Zn	0.40	<b>0.42***</b>	<b>0.62*</b>	<b>0.37**</b>	0.27	<b>0.33**</b>	-0.17	-0.13	0.03	0.01
<i>Env. micro-minerals</i>										
Li	-0.26	-0.19	0.16	0.05	-0.34	<b>-0.24*</b>	<b>0.64*</b>	<b>0.40***</b>	0.27	<b>0.21*</b>
B	<b>-0.68**</b>	<b>-0.34**</b>	<b>-0.61*</b>	<b>-0.34**</b>	<b>-0.63*</b>	<b>-0.27*</b>	<b>-0.54*</b>	<b>-0.21*</b>	-0.23	-0.15
Al	0.16	0.15	0.02	0.19	0.17	0.08	-0.08	0.10	0.30	<b>0.25*</b>
Ti	0.01	-0.06	-0.22	-0.11	0.07	-0.04	-0.42	-0.17	-0.01	0.09
Ni	<b>-0.58*</b>	<b>0.26*</b>	-0.40	-0.06	<b>-0.54*</b>	0.03	0.16	0.18	0.25	0.16
Sr	-0.18	-0.05	-0.13	0.05	-0.17	-0.10	0.49	<b>0.26*</b>	<b>0.54*</b>	<b>0.29*</b>

Sn	0.16	0.15	0.22	0.14	0.13	0.14	0.34	0.11	-0.18	-0.10
Ba	-0.49	0.01	-0.39	0.06	-0.47	-0.06	-0.05	0.17	0.45	<b>0.25*</b>
Pb	-0.48	<b>-0.26*</b>	<b>-0.57*</b>	<b>-0.42***</b>	-0.39	-0.12	<b>-0.59*</b>	<b>-0.47***</b>	<b>-0.55*</b>	<b>-0.38**</b>

\* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$

**Table 5.** farm/date ( $r_F$ ) and animal within farm/date ( $r_A$ ) correlation coefficients of color traits with latent explanatory factors and with individual essential and environmental macro- and micro- minerals concentration in meat

	<b>L*</b>		<b>a*</b>		<b>b*</b>		<b>C*</b>		<b>H*</b>	
	<b>r<sub>F</sub></b>	<b>r<sub>A</sub></b>	<b>r<sub>F</sub></b>	<b>r<sub>A</sub></b>	<b>r<sub>F</sub></b>	<b>r<sub>A</sub></b>	<b>r<sub>F</sub></b>	<b>r<sub>A</sub></b>	<b>r<sub>F</sub></b>	<b>r<sub>A</sub></b>
<i>Latent factors</i>										
F1 <i>Quantity</i>	0.47	<b>0.26*</b>	<b>0.73**</b>	<b>0.49***</b>	<b>0.61*</b>	<b>0.38**</b>	<b>0.71**</b>	<b>0.47***</b>	-0.31	<b>-0.23*</b>
F2 <i>Na+Fe+Cu</i>	-0.09	-0.06	0.23	<b>0.22*</b>	0.05	0.10	0.16	0.18	-0.40	<b>-0.29*</b>
F3 <i>K-B-Pb</i>	-0.24	<b>-0.26*</b>	-0.24	-0.08	-0.15	-0.04	-0.21	-0.07	0.19	0.04
F4 <i>Fe+Mn</i>	-0.48	<b>-0.22*</b>	0.20	<b>0.22*</b>	-0.06	0.03	0.10	0.15	-0.45	<b>-0.37**</b>
F4 <i>Zn</i>	-0.53	-0.19	0.36	<b>0.39**</b>	-0.02	0.11	0.22	<b>0.29*</b>	<b>-0.68**</b>	<b>-0.56***</b>
<i>Macro-minerals</i>										
Na	0.34	0.12	<b>0.64*</b>	<b>0.37**</b>	0.48	<b>0.27*</b>	<b>0.60*</b>	<b>0.35**</b>	-0.32	<b>-0.22*</b>
Mg	0.41	0.16	<b>0.64*</b>	<b>0.34**</b>	0.53	<b>0.29*</b>	<b>0.63*</b>	<b>0.33**</b>	-0.28	-0.15
P	0.44	0.20	<b>0.70*</b>	<b>0.47***</b>	<b>0.57*</b>	<b>0.39**</b>	<b>0.68*</b>	<b>0.47***</b>	-0.31	<b>-0.22*</b>
S	0.20	0.09	<b>0.79**</b>	<b>0.54***</b>	<b>0.54*</b>	<b>0.38**</b>	<b>0.72**</b>	<b>0.50***</b>	<b>-0.54*</b>	<b>-0.40**</b>
K	-0.10	-0.13	-0.06	0.05	-0.11	0.04	-0.08	0.06	-0.05	-0.10
Ca	-0.07	-0.01	0.11	0.08	0.15	0.001	0.14	0.05	0.04	-0.16
<i>Ess. micro-minerals</i>										
Cr	0.31	0.13	-0.08	-0.05	0.02	0.05	-0.04	-0.01	0.11	0.14
Mn	-0.05	-0.12	<b>0.54*</b>	<b>0.39**</b>	0.38	0.20	0.50	<b>0.33**</b>	-0.30	<b>-0.38**</b>
Fe	-0.35	<b>-0.31**</b>	<b>0.63*</b>	<b>0.63***</b>	0.11	0.16	0.44	<b>0.46***</b>	<b>-0.92***</b>	<b>-0.88***</b>
Cu	0.23	-0.01	<b>0.57*</b>	<b>0.35**</b>	0.51	0.16	<b>0.57*</b>	<b>0.29*</b>	-0.20	<b>-0.35**</b>
Zn	-0.44	-0.17	0.52	<b>0.53***</b>	0.09	<b>0.21*</b>	0.37	<b>0.41***</b>	<b>-0.79***</b>	<b>-0.65***</b>
<i>Env. micro-minerals</i>										
Li	-0.19	-0.06	-0.38	<b>-0.23*</b>	-0.43	-0.18	-0.42	<b>-0.21*</b>	-0.08	0.07
B	0.11	0.16	0.16	0.01	0.09	-0.06	0.14	-0.03	-0.10	-0.05
Al	0.43	0.10	<b>0.63*</b>	<b>0.36**</b>	<b>0.65*</b>	<b>0.35**</b>	<b>0.67*</b>	<b>0.38**</b>	-0.08	-0.10
Ti	<b>0.58*</b>	<b>0.28*</b>	<b>0.73**</b>	<b>0.37**</b>	<b>0.68*</b>	<b>0.39**</b>	<b>0.74**</b>	<b>0.40***</b>	-0.21	-0.07
Ni	-0.06	0.15	-0.32	0.13	-0.22	-0.18	-0.29	-0.05	0.18	0.04

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Sr	0.14	-0.04	-0.48	-0.17	-0.13	-0.11	-0.35	-0.15	<b>0.62*</b>	0.11
Sn	-0.39	-0.18	<b>-0.62*</b>	<b>-0.37**</b>	<b>-0.54*</b>	<b>-0.33**</b>	<b>-0.62*</b>	<b>-0.37**</b>	0.24	0.14
Ba	0.34	-0.09	0.16	0.10	0.27	0.001	0.21	0.07	0.12	-0.18
Pb	0.09	<b>0.26*</b>	-0.08	-0.08	-0.10	-0.12	-0.10	-0.11	0.03	0.03

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\* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$



**Table 6.** Farm/date ( $r_F$ ) and animal within farm/date ( $r_A$ ) correlation coefficients of some quality traits with latent explanatory factors and with individual essential and environmental macro- and micro-minerals concentration in meat

Elements	pH		Cooking loss, %		Shear force, N/cm <sup>2</sup>	
	$r_F$	$r_A$	$r_F$	$r_A$	$r_F$	$r_A$
<i>Latent factors</i>						
F1 Quantity	0.05	0.04	0.08	0.03	-0.32	-0.10
F2 Na+Fe+Cu	-0.08	0.11	-0.10	0.03	-0.09	0.01
F3 K-B-Pb	-0.14	0.13	-0.53	<b>-0.47***</b>	-0.06	0.01
F4 Fe+Mn	0.15	0.20	-0.33	-0.11	-0.32	-0.11
F4 Zn	0.10	-0.07	-0.44	-0.20	-0.42	-0.20
<i>Macro-minerals</i>						
Na	0.01	-0.04	-0.07	0.08	-0.27	-0.02
Mg	0.01	0.06	0.01	-0.04	-0.27	-0.03
P	0.02	0.08	0.03	-0.02	-0.32	-0.06
S	-0.01	0.04	-0.16	<b>-0.22*</b>	-0.48	-0.15
K	-0.20	0.06	<b>-0.61*</b>	<b>-0.42***</b>	-0.23	-0.02
Ca	-0.47	-0.03	-0.22	-0.12	-0.29	-0.10
<i>Ess. micro-minerals</i>						
Cr	-0.15	-0.01	-0.21	-0.18	-0.11	-0.05
Mn	0.22	0.12	-0.13	-0.10	0.08	0.03
Fe	0.15	0.15	-0.36	-0.14	-0.42	-0.07
Cu	0.08	0.13	0.14	0.09	-0.06	0.12
Zn	0.08	0.02	-0.49	<b>-0.24*</b>	-0.49	-0.19
<i>Env. micro-minerals</i>						
Li	0.01	0.01	-0.46	-0.11	-0.17	-0.01
B	0.18	-0.20	0.46	<b>0.31**</b>	0.25	-0.08
Al	0.17	0.06	-0.02	-0.18	-0.32	-0.08
Ti	0.24	0.07	0.18	0.02	-0.30	-0.09
Ni	-0.15	0.12	0.06	-0.01	0.49	-0.06
Sr	<b>-0.62*</b>	-0.13	0.05	-0.05	-0.02	-0.01
Sn	-0.05	0.01	-0.11	-0.13	0.20	0.08
Ba	-0.36	0.00	0.16	-0.07	-0.20	-0.08
Pb	0.10	-0.20	0.53	<b>0.38**</b>	0.30	-0.03

\* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$

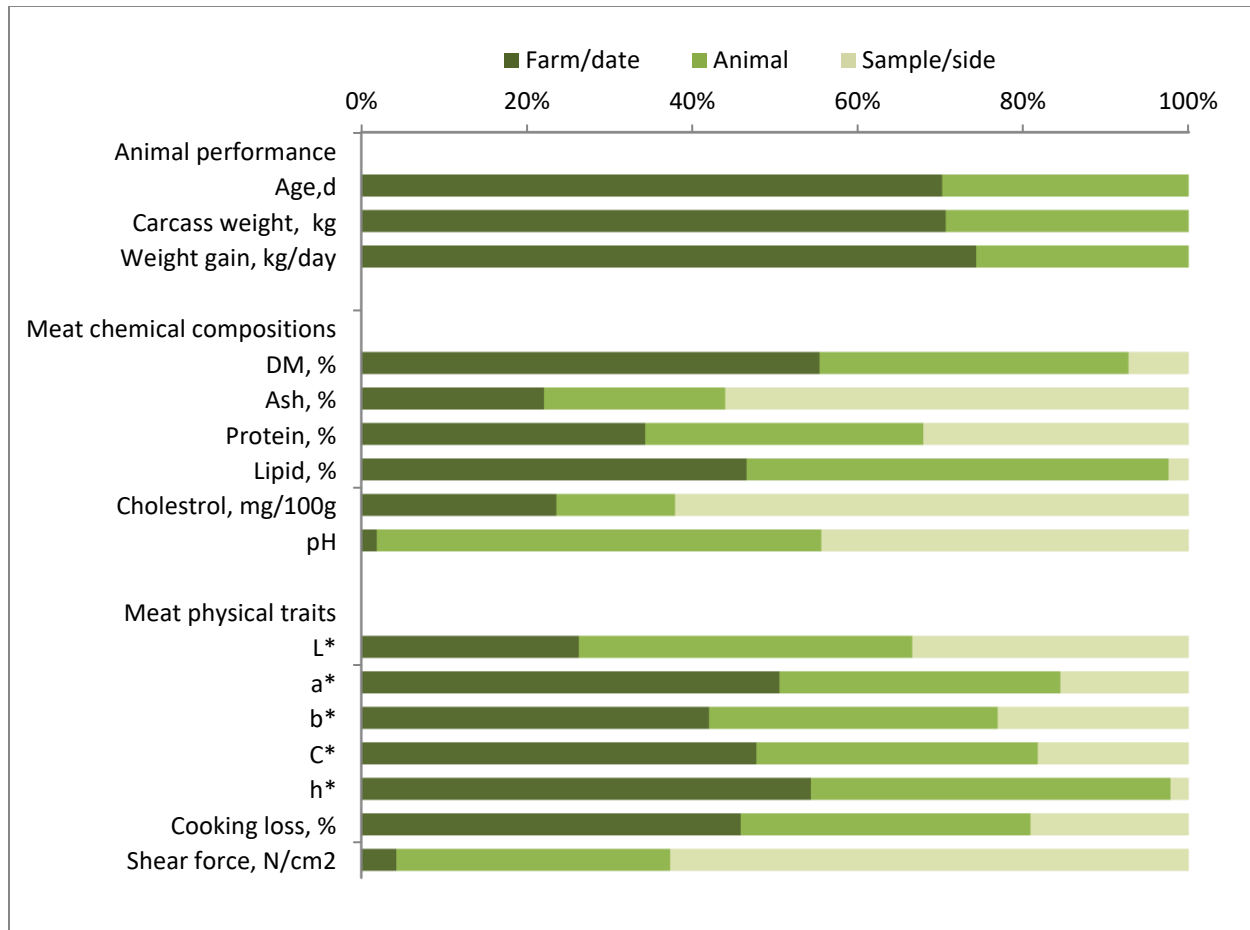
1 **Figure captions**

2 **Figure 1.**

3 Percentage incidence of farm/date of slaughter, animal within farm/date, and sample/side within  
4 animal (residual) variances on total phenotypic variance of animal performance and meat quality  
5 traits.

6

7

8 **Figure 1.**

9