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**Associations among animal, carcass, muscle characteristics, and fresh meat color traits in
Charolais cattle**

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Abstract

This study investigated the effects of animal, carcass and muscle characteristics on initial color traits of steaks from 887 Charolais cattle. First, the fixed factors of year of birth, experiment and sex had strong impacts on color traits. From the covariates, increased age lead to intense color (low h^* , -1.55 units) and darker and vivid meat (high a^* , b^* and C^* : $+4.56$, $+3.41$ and $+5.61$, respectively). Increases in fatness score and carcass fat weight were associated with increases in a^* , b^* and C^* (redness; $+2.90$ to $+4.06$ for a^* ; yellowness; $+2.60$ to $+3.76$ for b^* ; and vividness, $+3.87$ to $+5.49$ for C^*) and a darker colored lean (L^* ; -1.56 to -3.23). As pH_{24h} increased, a^* (less red) and C^* (less vivid) decreased (-3.06), whereas hue angle increased ($+2.69$) leading to poorer color. The selection of animals for high degree of muscularity or slaughter weight resulted in lighter and darker meat, respectively. The studied covariates could be used as indicators of Charolais beef color traits.

Keywords: Beef color; Sex; Animal and carcass properties; Carcass Grading; Chemometrics.

1. Introduction

Visual appearance of beef such as color, marbling and package purge are important quality cues for consumers (Font-i-Furnols & Guerrero, 2014). Irrespective of the cultural aspects, experience or consumption habits, fresh beef color is the first visual cue evaluated by consumers and is probably the greatest quality trait that drive consumer decision of meat purchase at the point of sale (Mancini & Hunt, 2005). Meat color is influenced by the concentration and physicochemical state of myoglobin (Mb), a sarcoplasmic heme protein (Mancini, 2009; Mancini & Hunt, 2005; Neethling, Suman, Sigge, Hoffman, & Hunt, 2017). Furthermore, Mb content and hence color intensity, depends not only on the muscle but also on several factors that work together to influence color stability, which can be categorized into intrinsic and extrinsic factors (Gagaoua, Monteils, Couvreur, & Picard, 2017; Gagaoua, Terlouw, & Picard, 2017; Neethling et al., 2017). The intrinsic factors can be attributed to breed, sex, age, contractile and metabolic muscle properties, muscle composition and characteristics such as connective tissue content, intramuscular fat, pH and others. The extrinsic factors are mainly related to the rearing factors, transport and animal handling, post-slaughter ageing conditions and to the commercial practices and conditions during retail display such as meat cuts, additives, temperature, packaging and type of light. These factors and/or their interactions are all reported to affect fresh meat color (Mancini & Hunt, 2005; Neethling et al., 2017).

However, there is a paucity in the studies that have investigated the relationships between fresh beef color traits with different intrinsic and extrinsic factors in the same study, namely animal and carcass phenotypes. In beef, the relationships between animal and carcass characteristics were specifically addressed in the context of dark cutting incidence of meat under the Meat Standards Australia grading system (McGilchrist, Alston, Gardner, Thomson, & Pethick, 2012) and more recently under the Canadian grading system (Mahmood, Basarab, Dixon, & Bruce, 2016). Both studies have approached the overall rearing factors that would identify groups of carcasses with dark cutting incidence and causative effects of this technological defect but without direct description of relationships and impact on instrumental color traits of fresh beef. Furthermore, most of the earlier studies had generally been part of broader investigations on meat quality and were not specifically focused on the robust relationships with beef color and few of them deal with carcass characteristics.

In this work, we hypothesized that the rearing factors and carcass characteristics will play a more pivotal role than muscle properties on fresh beef color traits measured 24 hours *post-*

mortem. Thus, the aim of the present study is to seek using at the same time chemometrics and multivariate regression analyses the relationships that exist between rearing factors, animal, carcass and muscle characteristics with initial beef color traits from individual data of Charolais cattle, the predominant French meat breed.

2. Materials and methods

2.1. Experiments, animals handling and slaughtering

Data were collected from 887 purebred Charolais cattle, all reared and managed in the French INRA institute (National Institute for Agricultural Research). Data from 27 independent experiments were compared using 13 experiments of 446 young bulls; 8 experiments of 223 steers, and 6 experiments of 218 cows. Young bulls and cows were reared in INRA-UE232 (Bourges) experimental site and steers in INRA-UE326 (Pin-au-Haras). The animal's genetics reflect the distribution of the major animal types of the Charolais breed slaughtered in France. All the animals were reared and kept in accordance with the French animal protection regulations defined in the French law (Code Rural, articles R214-64 to R214-71; Legifrance, 2016). The animals were all born from mature cows, weaned at 32 weeks and then kept in an open shed and fed a pelleted diet *ad libitum* with a limited amount of cereal straw (mainly barley, wheat and/or oats at a percentage of ~12%) until slaughter. The composition of the diet was consistent across years. The pellets were composed of approximately 57% alfalfa hay and 37% corn with cane molasses and urea to balance the diet. All cattle were growth-promotant free other than the obvious hormone differences for the three cattle sexes.

Before slaughter, all animals were fasted for 24 h and had free access to water. The animals were slaughtered under the standard conditions in the same experimental slaughterhouse of INRA at the Auvergne-Rhône-Alpes Research Center (previously INRA-UERT, and now INRA-Herbipôle, Theix). The animals were stunned using a penetrative captive bolt, prior to exsanguination. Slaughtering was performed in compliance with French welfare regulations set at the level of European Directive (2001/88/EC). The carcasses were not electrically stimulated and they were stored at a maximum temperature of 4°C (between 2 and 4°C) until 24 h *post-mortem* according to standard commercial practice.

2.2. Animal and carcass characteristics determination

Animal age and slaughter and hot carcasses weights (2h *post-mortem*) were recorded systematically for all the animals. Carcass weight is the weight of the animal after the removal

of its head, hide, feet/legs, thoracic organs, internal fats, and abdominal organs. Carcass conformation was graded under the EU beef carcass classification (EUROP) scheme. This was done at the slaughterhouse using experts familiar with the EUROP grid (Commission Regulation (EC) 1249/2008). Under EUROP grid, five conformation classes are defined, represented by the letters E, U, R, O, and P. The scoring consists of a visual assessment of carcass muscling where carcasses graded as 'E' have the most muscularity, and this decreases through to 'P' which have the least muscularity. European Union regulations allow for 3 subdivisions of each conformation, high: "+", medium: "±" and low: "-". Hence, an incremental scale ranging from 1 to 15 was used, where 1 corresponds to P- (very low muscle development) and 15 to E+ (very high muscle development). At the same time, the degree of fat cover of the carcasses (hereafter fatness score) which describes the amount of fat on the outside and the inside of the carcass was numerically scored from 1= leanest to 5 = fattest according to the same European classification (Commission Regulation (EC) 1249/2008).

2.3. Carcass composition by the 6th rib dissection

The entire 6th rib at the margins of the 5th and 7th ribs was excised from the right side of each carcass and weighed. The whole carcass composition was estimated from the composition of the 6th rib determined after physical dissection following standard commercial practice involving a close trimming and deboning of the muscle as shown in **Fig. 1**. The physical dissection of the ribs into fat, muscle and bone is the reference method to assess the composition of carcasses (Robelin & Geay, 1975). The total dissected weights of muscle (LT muscle and other muscles), bone (bone and other tissues likely ligaments, tendons and cartilages) and fat (both subcutaneous and intermuscular fat) were recorded by this method (**Table S1**). After that, the prediction equations proposed by (Robelin & Geay, 1975) for Charolais breed were used to compute the carcass muscle and carcass fat weights, in kg.

2.4. pH_{24h} determination

For muscle pH at 24h *post-mortem*, the measurements were conducted by inserting a glass electrode on five different locations per steak using a Hanna HI 9025 pH/ORP meter suitable for meat penetration (Hanna Instruments Inc., Woonsocket, RI, USA). The pH meter was first calibrated at chilling temperature using pH 4 and pH 7 buffers. Then, the pH was measured in the *Longissimus thoracis* muscle (LT, fast oxido-glycolytic) between the 6th and 7th rib. The five measurements within each steak were averaged for statistical analyses.

2.5. Meat color measurements

For determination of the initial meat color (not color stability), the protocol described by (Gagaoua, Monteils, et al., 2017; Gagaoua, Terlouw, et al., 2017) was used. Briefly, a Minolta colorimeter (Minolta CR400, Konica Minolta, Japan) was used to measure objective meat color 24 h after slaughter using illuminant D65 (Daylight at noon), 8 mm diameter aperture and a 10° standard observer. The same colorimeter was standardized prior color determination using the ceramic white tile as per the directions of the instrument manual.

Fresh ribeye slices were cut perpendicular to the LT muscle to be not less than 2.5 thick. The slices were placed on a polystyrene tray and overwrapped with highly plasticized polyvinyl chloride (0.13 mm thick). Packaged slices were bloomed for 1h at 4°C and then color scans were collected under the CIE-LAB system. L^* (lightness) is measured from black to white, a^* (redness) has a negative value for green and a positive value for red and b^* (yellowness) values have a negative value for blue and a positive value for yellow. Six measurements were taken per slice, and the averages were used in the statistical analysis.

Chroma (C^*) and hue angle (h^*) were then calculated using the following equations in AMSA, (2012). Larger C^* values indicate greater red color intensity and larger h^* values indicate a less red and more discolored lean.

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

$$h^* = [(b^*/a^*) \tan^{-1}] \quad (2)$$

2.6. Statistical analyses

The individual data from the 887 Charolais cattle including three sexes (young bulls, steers and cows) were analyzed using SAS statistical software (SAS 9.3, SAS Institute INC, Cary, NC, USA) and XLSTAT 2017.19.4 (AddinSoft, Paris, France). Before analysis, raw data means were scrutinized for data entry errors and outliers from the general trends and these were excluded from the analyses, or adjusted when the cause of the outlier could be identified. This was conducted by subjecting data to Smirnov–Grubb's outlier test at 5% levels. After this, a general description analysis was conducted by computing size, means, standard deviations and minimum and maximum ranges of color traits and covariates data (**Table 1**).

Principal component analysis (PCA) was performed by projecting the color traits as active variables and the Charolais sex as supplementary data to highlight the associations between the loadings of the variables. PCA is a projection method that allows an easy visualization of all the information contained in a dataset. In addition, this multivariate analysis allows to find

out in what respect groups of animals are different from others and which variables contribute most to these differences. In this study, PCA was further performed to visualize the relationship between the covariates data (animal, muscle and carcass characteristics) with color traits and their distribution according to Charolais sex. To check the suitability of the factorial model, the Kaiser-Meyer-Olkin (KMO) test for sampling adequacy was used and the overall KMO values are reported for each PCA. Furthermore, only the components with eigenvalues greater than 1.00 were retained for interpretation. An eigenvalue represents the amount of variance that is captured by a given component. Eigenvalue criterion is one of the most commonly used criteria for solving the number of components problem, also known as the Kaiser-Guttman criterion (Kaiser, 1974).

For the study of the robust relationships, data were initially analyzed to create a base (core) model for each color trait (Bonny et al., 2015; Calnan, Jacob, Pethick, & Gardner, 2016). Briefly, the multivariate regression analysis under SAS software was used. It allowed us to consider the fixed effects for year of birth, experiment, sex and their interaction for each color coordinate. Terms in the model and their first order interactions were removed in a stepwise fashion if non-significant ($P > 0.05$). After base models preparation, the animal (age and slaughter weight), carcass (EUROP conformation score, carcass fatness score, hot carcass weight (HCW), carcass fat and carcass muscle weights) and muscle characteristics (pH24h) were included in the base models of the color traits as covariate using multivariate regressions. For carcass fat and carcass muscle weights covariates, the predicted values from the equations described by (Robelin & Geay, 1975) were used. The base models allowed testing the magnitude of covariates effects on each beef color trait one by one (one at a time). The magnitude of each fixed effect on ribeye steaks color traits were then expressed as the unit difference between minimum and maximum predicted means for L^* , a^* , b^* , C^* and h^* color traits between different fixed rearing factors (**Table 2**). For all terms in the models, the curvilinear response was also assessed but none was significant, hence, only linear relationships are given. The magnitude of covariate effects on L^* , a^* , b^* , C^* and h^* are expressed as the unit change in their prediction across the range of each animal, carcass or muscle covariates.

Finally, for a better characterization of the relationships identified by multivariate regression analysis, another chemometric analysis was used, namely principal component regression (PCR). PCR is a regression method that can be divided into three steps: the first step is to run a PCA on the table of the explanatory variables, herein all the covariates with

the dependent variable (color traits one by one). The second step is to run an Ordinary Least Squares regression (OLS regression) on the selected components. At the end, to compute the parameters of the model that correspond to the input variables (Gagaoua, Bonnet, Ellies-Oury, De Koning, & Picard, 2018). In our case, PCRs were performed to generate predictive models using the optimal number of components from all the covariates for each color coordinate at one time. The basic idea behind PCR is to calculate the principal components and then use some of these components as predictors in a linear regression model fitted using the typical least squares procedure (Gagaoua et al., 2018; Jolliffe, 1982). To select PCs (from the few chosen PCs) for regression, we used the forward selection approach. At each stage of selection process, a test was run after a new PC was added to check if some PCs could be deleted without considerably increasing the root mean square error (RMSE). This selection process ended when a minimum RMSE was obtained. Besides the percentage of variance explained, the eigenvalues of the PCs can be of use to decide how many PCs to include in the PCR of the PCs on the Y -variable (each color coordinate). The best model was regarded as the final forecast.

3. Results and Discussion

3.1. Presentation of the dataset of this study

An overview of the dataset used (**Table 1**) and the statistical analyses applied at each step are presented. The data of the Charolais cattle showed wide variations in the properties of interest referring to color traits (L^* , a^* , b^* , C^* and h^*). Large variations also occurred for age and slaughter weights of the animals, and in their carcass characteristics (EUROP conformation score, carcass fatness score, hot carcass weight, carcass fat weight and carcass muscle weight). However, despite the large range of the values of pH24h of the LT muscle from different Charolais sexes (5.10 – 6.07), the coefficient of variation is only 1.78%. These data were then used as follows:

i) projection of the color coordinates in a first principal component analysis (PCA) (**Fig. 2**) to highlight the relationships between the color traits according to the sex of the Charolais animals (young bulls, steers and cows);

ii) analysis of variance for sex effect on both color traits and covariates data (**Table 2**) to perform comparisons between young bulls, steers and cows;

iii) projection in a new PCA of the color traits with animal, muscle and carcass characteristics covariates (**Fig. 3**) to highlight the relationships according to young bulls, steers and cows;

iv) computing base models on the color data by taking in account the rearing factors and production factors (year of birth, experiment and sex) by the means of multivariate regression analyses (**Table 3**) and then to test in a stepwise manner the effect of the covariates in the models (**Fig. 4**);

v) finally, principal component regression analysis (**Fig. 5**) was performed to assess the potential of all the covariates to be used at the same time as explanatory variables of each color trait and propose prediction equations (Gagaoua et al., 2018).

3.2. Color traits for three sexes of Charolais cattle

Irrespective of the year of birth, experiment, sex and the interaction of these factors, the PCA allowed checking without significant loss of information differences among the three sexes of Charolais cattle (**Fig. 2**). Thus, the PCA run on the five instrumental color parameters gave two axis with eigenvalues of 3.3 and 1.1, respectively (**Fig. 2**). These first two axes explained 89.4% of the variability with eigenvectors greater than 0.5. The first PC accounting for 66.7% of variability was positively related with redness (a^*), yellowness (b^*) and Chroma (C^*) and negatively with lightness (L^*). On the second PC accounting for 22.7% of the variability, hue angle (h^*) was exclusively projected alone in the positive side. The PCA allowed the separation between the three Charolais sexes according to their color traits (**Fig. 2**).

The bi-plot highlighted that the animals characterizing dark and vivid meat with the highest C^* -values were loaded on the positive side of the first PC (**Fig. 2**). The C^* index represents the color intensity and is a good blooming indicator in fresh meat exposed to oxygen. The animals with high a^* , b^* and C^* -values grouped mainly those of cows as confirmed by the mean values given in **Table 2**. In comparison to young bulls, a^* , b^* and C^* -values of cows showed respectively +6.72, +4.75 and +8.09 units difference. Inversely to cows, on the negative side of the first PC are loaded the young bulls, characterizing light red meat by their highest L^* -values (+7.46 units compared to the cows). The steers, characterizing intermediate values for the five color traits in comparison to the two other Charolais sexes (**Table 2**) are mainly loaded on the center and positive side of the first PC (**Fig. 2**). This study agrees with earlier experiments indicating differences in physicochemical properties of meat

as a function of sex (Gagaoua et al., 2016; Page, Wulf, & Schwotzer, 2001; Priolo, Micol, & Agabriel, 2001). However, it is worth mentioning that the impact of sex on muscle color of cattle has been scarcely studied and most of them are inconclusive. Our results highlighted that the color of cow's fresh meat was brighter and vivid as compared with young bull's fresh meat. It could be due to the increased intramuscular fat content of cows as it decreases lightness of meat color (Boccard et al., 1979; Węglarz, 2010; Wulf & Wise, 1999). The impact of the factors related to animal, carcass and muscle are discussed below (see section 3.4), covariate by covariate, using the base models built for each color trait.

3.3. Animal and carcass characteristics for three sexes of Charolais cattle

The comparisons in animal, muscle and carcass characteristics between the three sexes of Charolais cattle are presented in **Table 2**. All the covariates were significantly different ($P < 0.01$). Steers and cows Charolais were heavier than young bulls. This is maybe due to the longer feeding period (Venkata Reddy et al., 2015). In France, young bulls are generally slaughtered at 17 months of age (>8 and <24 months), whereas steers are slaughtered after 24 months old ($24 < \text{age} < 36$). These are in accordance to the continental cattle characteristics known as larger, leaner and more muscular; which lead to heavier hot carcass weights than British breeds. In this study, even though the slaughter and hot carcass weights are slightly less in young bulls, the EUROP conformation score and muscle carcass weight are high than those of steers and cows. This particularity for young bulls would be explained by their greater growth rate caused by the gradual increase in the secretion of anabolic hormones, namely testosterone, and other muscle building hormones during the growth period (Velloso, 2008). This is in line with the hypothesis stating that steers and young bulls would reflect growing cattle, whereas cows would typically reflect mature cattle (Venkata Reddy et al., 2015). Therefore, the differences in weight could result from compensatory gains/nutritional status in mature cattle, whereas differences in carcass attributes in young bulls could be growth-related (Oksbjerg & Therkildsen, 2017; Purslow, 2017).

Carcass fatness scores ranged from 2.48 (young bulls) to 3.50 (cows) on a 5-point scale. The highest fatness scores (3.5 points with +1.02 points than young bulls) and carcass fat weight in kg (+24% than young bulls) found for cows agree with their capacity to deposit fat. Earlier study reported that intramuscular fat is the last to be deposited and female cattle possess high levels of the genes that control the deposition process (Lee, Evans, Nute, Richardson, & Scollan, 2009). Otherwise, steers have higher carcass fatness scores and carcass fat weight than bulls, which is mainly attributable to the lower production of

testosterone by steers (Prado et al., 2009). Overall, it has been reported in earlier studies that entire males' carcasses had higher growth and lower quantity of fat compared to females (Daza, Rey, Carrasco, & López-Bote, 2012).

The pH_{24h} values for the three sexes of Charolais cattle are given in **Table 2**. The effect of sex on pH_{24h} values can be partly related to the reactivity of the animals to pre-slaughter handling, which can be different between males (young bulls and steers) and females (cows). Bulls are known as more stressed animals, and with glycogen depletion, the pH does not decrease as it does in cows (Page et al., 2001; Węglarz, 2010). Stress reactions may influence *post-mortem* muscle metabolism and consequently, meat quality traits including pH. In this study, pH_{24h} was greater than 5.8 and 6.0 for 1.8% and 0.11% of cattle, respectively. In agreement to our results, a study by (Kreikemeier & Unruh, 1993) reported that the incidence of pH greater than 5.8 was 1.7%. In France, (Mounier, Dubroeuq, Andanson, & Veissier, 2006) reported that ultimate pH of *Longissimus thoracis* muscle of bulls greater than 6.0 was 3.36%. In contrast to our results, a Spanish study showed that the incidence of pH greater than 5.8 and 6.0 were 13.89% and 4.02% respectively on the same muscle of 5494 cattle including both female and male (Mach, Bach, Velarde, & Devant, 2008). These authors stated that the differences among studies could be attributed to the different rearing and finishing practices. In the present study, most of the carcasses fell in a very narrow range with respect to muscle pH and 90.85% of them were in the range of 5.40 to 5.70, a range considered as the "normal" ultimate pH for beef *Longissimus* muscle following normal *post-mortem* metabolism (Matarneh, England, Scheffler, & Gerrard, 2017; Page et al., 2001). Since the extent of *post-mortem* metabolism was proposed to be a function of muscle glycogen content at the time of slaughter (Matarneh et al., 2017), various factors would also affect the differences in pH_{24h} between animal types, including breed and sex (Gagaoua et al., 2016).

The above-described parameters, *i.e.*, color traits and covariate data, were projected in a new PCA (**Fig. 3**). The three sexes of Charolais cattle were loaded as in the first PCA given in **Fig. 2**: cows at the right, young bulls at the left and steers on the center of the first PC characterized by an eigenvalue of 5.1. On the positive side of the first PC, are loaded a^* , b^* and C^* traits with age at slaughter, carcass fatness score and carcass fat weight and on the negative side, L^* was loaded with conformation scores. On the positive side of the second PC with an eigenvalue of 2.9, are mainly loaded carcass muscle weight and hot carcass weights. This PCA shows that meat color traits would be linked to certain variables related to animal, carcass and muscle characteristics. This is in agreement with the earlier studies reporting that

several factors would be related with fresh meat color in ruminants, including carcass characteristics (Calnan et al., 2016; Priolo et al., 2001), feeding diets (Priolo et al., 2001), muscle pH (Calnan et al., 2016; Page et al., 2001), muscle fat content (Farouk & Lovatt, 2000), physical activity (Priolo et al., 2001) and many others (Gagaoua, Couvreur, Le Bec, Aminot, & Picard, 2017; Gagaoua, Monteils, et al., 2017). The relationships between the covariate data investigated in this study and meat color traits are developed in the following sections according to the base models generated after removing the effects of year of birth, experiment, sex and their interactions. The associations were also addressed by the means of principal component regression by including the 8 covariates at the same time for each color coordinate.

3.4. Associations among animal, carcass and muscle characteristics with beef color traits

The magnitude differences from the base models by the minimum and maximum mean L^* , a^* , b^* , C^* and h^* values between the different fixed production effects are shown in **Table 3**. They allowed highlighting the effect of animal and carcass characteristics on fresh meat color traits. It is recognized that *ante-mortem* factors can influence two aspects of meat color, namely overall muscle brightness/darkness and color stability (Mancini, 2009; Mancini & Hunt, 2005; Neethling et al., 2017; Page et al., 2001). The year effect showed for all the color traits a magnitude difference higher than 9.0 units and the greatest difference was calculated for C^* (Mag = 13.78; $P < 0.001$). This means that we would expect in the future to consider the year effect on meat color coming from Charolais breed by adding 13.78 units of differences when addressing C^* -values. However, the interpretation of year effect is complex but agrees with the findings on lamb by (Calnan et al., 2016). For instance, we may also suppose that the year effect is confounded with seasonal differences effects. Finally, the interaction between the three production factors showed great impact on the five color traits. Among them, L^* was the strongly affected trait by the interactions with a magnitude difference of 18.92 units.

3.4.1. Age at slaughter

The effect of age at slaughter as a covariate in this study is to some extent confounded with the sex effect. To avoid this and further rearing factors, base models were used to highlight the real and residual variability within each color trait. Strong relationships of age at slaughter were found with the five color coordinates, negatively on L^* and h^* and positively on a^* , b^* and C^* (**Fig. 4**). These means that as increased age did, L^* get lighter, h^* lower (intense

color) and a^* , b^* and C^* darker and vivid. As h^* measurements provide a good indication of discoloration in meat, larger h^* -values due to decreased age would indicate more metmyoglobin formation (Neethling et al., 2017). Hence, by increasing the age at slaughter, the impacts in terms of units are in the following order: C^* (Mag = +5.61; $P < 0.001$), a^* (Mag = +4.56; $P < 0.001$), L^* (Mag = -4.01; $P < 0.001$), b^* (Mag = +3.41; $P < 0.001$) and weakly on h^* (Mag = -1.55; $P < 0.05$). The brown meat color becomes noticeable to the consumers when an approximate of 60% of the myoglobin is converted to metmyoglobin (Murray, 1989). Thus, it is not so sure whether the weak but significant differences found in the h^* -values would be apparent to the consumer. Numerous studies indicated that the slaughter age of farm animals determines the physicochemical characteristics of their meat (Calnan et al., 2016; Neethling et al., 2017; Page et al., 2001). Regarding cattle and in agreement to our findings, darker meat by increasing the age at slaughter, namely from mature cows, has been previously observed (Boccard et al., 1979; McGilchrist et al., 2012). It was proposed that the lower L^* -value in beef is most likely caused by higher myoglobin contents, as the myoglobin concentration increases with age (Moon, Yang, Park, & Joo, 2006; Neethling et al., 2017). Also, the shift from the glycolytic to oxidative fiber types, resulting in higher concentration of muscle myoglobin with advancing maturity has been suggested as the main reason of darker beef in older animals (Moon et al., 2006). The work by (Calnan et al., 2016) on loin meat of 8165 lambs support our findings and the above cited works. In the present study, the lower h^* and higher a^* , meaning intense and darker meat, observed in cows (the inverse is true for young bulls) maybe also reflected by a higher myoglobin content (Moon et al., 2006). Changes in muscle protein profile in response to slaughter age would also be involved (Gulyás et al., 2015). We think that the changes in proteome muscle due to age at slaughter might explain a part of the variability of color traits (Gagaoua et al., 2015; Gagaoua, Terlouw, et al., 2017).

3.4.2. Slaughter and hot carcass weights

Slaughter and hot carcass weights are the two other covariates analyzed in the base models of the five color coordinates of Charolais cattle (**Fig. 4**). In most beef evaluation systems, carcass weight is a strong determinant of price received per animal and is therefore of great importance to beef producers for carcass classification (Monteils et al., 2017). The findings of this study showed that the increase in slaughter weight is associated with increase on C^* (Mag = +2.37; $P < 0.01$), a^* (Mag = +1.81; $P < 0.05$), and b^* (Mag = +1.54; $P < 0.05$), slight decrease on L^* (Mag = -1.86; $P < 0.05$), and no significant effect on h^* (Mag = +0.30; $P >$

0.05). These stress forward that selection of animals for high slaughter weight will have positive effect on darker meat (high a^* , b^* and C^* -values) and negative impact on L^* -values. However, no significant effect of hot carcass weight ($|0.17 < \text{Mag} < 0.85|$; $P > 0.05$) was found with any of the color traits. Thus, hot carcass weight is not suitable to be used by the stakeholders to manage Charolais beef color. Among the earlier studies, (Wulf, O'Connor, Tatum, & Smith, 1997) reported weak positive relationships between slaughter and hot carcass weights with L^* , a^* and b^* color traits of 317 cattle. In agreement to our findings, (Page et al., 2001) failed to found any relationships between hot carcass weight and color traits. However, these authors reported that lean maturity was related with L^* , a^* and b^* values. Similarly, it is noteworthy that heavier-muscled carcasses tended to have a much higher incidence of lightness/brightness than normal muscle (Murray, 1989). Previous data in bulls showed that increased slaughter weight and growth rate increased carcass pH and reduced L^* values (Vestergaard, Oksbjerg, & Henckel, 2000). In lamb and inversely to the results of the present study, (Calnan et al., 2016) found strong positive effect of hot carcass weight on a^* , b^* , C^* and h^* . The contrasts between lamb and beef may highlight differences in the underpinning mechanisms. The results of this study maybe partly related to maturity and pH decline (Marichal, Castro, Capote, Zamorano, & Argüello, 2003; Murray, 1989). For example, (Marichal et al., 2003) found a slight decrease of L^* with increased body weight and attribute it to the close relationship with changes in pH.

3.4.3. Conformation score and carcass muscle weight

The beef carcass grading system in France, as in the European Union, is based on the EUROP classification system. This system is a simple method of grading carcasses that describes the most commercially significant characteristics despite the need of additional indicators for better carcass grading (Monteils et al., 2017). Conformation score was in this study linked with meat color traits (**Fig. 4**). The hypothesis that conformation score would have an effect on beef color coordinates was supported by our findings. An increase in the conformation score is associated with a strong decrease on C^* ($\text{Mag} = -5.42$; $P < 0.001$), a^* ($\text{Mag} = -3.95$; $P < 0.001$), b^* ($\text{Mag} = -3.75$; $P < 0.001$), increase on L^* ($\text{Mag} = +2.50$; $P < 0.001$) and no significant effect on h^* ($\text{Mag} = -0.25$; $P > 0.05$). In other words and inversely to age at slaughter, selection of animals on their high degree of muscularity will lead to lighter beef color with higher L^* -values and lower redness and Chroma, respectively. The association between conformation score and color traits is maybe a result of muscle growth and development, and hence of lean maturity which may favor less fat deposition known to be

related with red meat (Farouk & Lovatt, 2000). Indeed, it is believed that EUROP conformation score has negative relationships with carcass fat proportion, carcass fatness score and intramuscular fat. For example, in Holstein-Friesians, an increase by one point in conformation score (15-point scale) was associated with a 4.40 g/kg reduction in fat carcass proportion (Conroy et al., 2009).

Carcass muscle weight was in this study slightly and negatively related with C^* (Mag = -2.30; $P < 0.01$) and a^* (Mag = -1.82; $P < 0.05$), respectively (**Fig. 4**). These are in line with the findings described above for carcass conformation. The highest is carcass muscle weight, the lowest is a^* , and the clearer would be the color of the meat. This confirms the findings discussed above if a comparison is done between cows and young bulls only (**Table 2**). These relationships would be partly a consequence of muscle fiber proportions and their characteristics, muscle development and inter-individual variability in fiber type composition (Astruc & Venien, 2017; Oksbjerg & Therkildsen, 2017). Fiber type proportions and eventual shift in muscle fiber type are known to differ considerably during animal growth and between lean and muscular animals (Oksbjerg & Therkildsen, 2017; Purslow, 2017). These dissimilarities may therefore be directly associated with variations in color due to differences in the concentration of heme pigments amount, especially myoglobin (Astruc & Venien, 2017).

3.4.4. Carcass fatness score and carcass fat weight

Carcass fatness score and carcass fat weight impacted significantly ($P < 0.05$) C^* , a^* , b^* and L^* (**Fig. 4**). The impact was in the same direction for both covariates. However, neither carcass fatness score nor carcass fat weight had a significant effect on h^* . An increase in fatness score was associated with a strong increase on traits characterizing dark-red and vivid meat by a^* (Mag = +2.90; $P < 0.001$), b^* (Mag = +2.60; $P < 0.001$) and C^* (Mag = +3.87; $P < 0.001$). Since C^* is computed using a^* and b^* , it follows that a beef cut with high fat content gave higher a^* and b^* that lead to higher C^* , hence indicating a more saturated or vivid color (the inverse is also true). Carcass fat weight was associated in the same trend with a strong increase on C^* (Mag = +5.49; $P < 0.001$), a^* (Mag = +4.06; $P < 0.001$), b^* (Mag = +3.76; $P < 0.001$) and a decrease on L^* (Mag = -3.23; $P < 0.001$). These means that as fat content did, L^* get lighter and a^* , b^* and C^* darker and vivid. These has important industry significance given the importance of the two covariates in other aspects of meat quality, namely tenderness, juiciness and flavor, known to influence consumer preferences (Font-i-Furnols & Guerrero, 2014). To our knowledge, the studies that has related fatness score with color

coordinates and other meat quality traits are very scarce. The most relationships were mainly with carcass fat weight or marbling scores. Hence, the discussion of the results of this study to those of the large literature were with marbling as it is known to be strongly related with EUROP fat score (Dow, Wiegand, Ellersieck, & Lorenzen, 2011). Marbling score was developed as an indicator of quality and palatability since it describes the amount and distribution of visible intramuscular fat tissues (Dow et al., 2011). The relationships identified in this study agree to our recent proposal to use marbling score as an indirect mean for meat sensory quality assessment (Monteils et al., 2017). The earlier works showed that higher levels of desirable fat in muscle or carcass lead to darker beef color (Boccard et al., 1979; Wulf & Wise, 1999). According to these results, we may suggest to use carcass fatness, as an easy parameter, to segregate carcasses according to their future color (dark and vivid meat) at the time of grading. On another hand, the negative associations between carcass fatness score and carcass fat weight parameters with L^* agree to our expectations and to those reported in the literature (Fiems et al., 2000). Similarly, earlier studies reported relationships between fat thickness of beef carcasses and muscle color (Page et al., 2001; Wulf et al., 1997). The associations maybe attributed to fat deposition during animal growth and especially during the fattening period (Farouk & Lovatt, 2000). Thus, one can suppose that managing the rearing factors during the finishing period of the animals (Gagaoua, Monteils, et al., 2017) would be of interest to get the desirable color of beef. This would be mainly possible, as it has been reported that fat deposition shows relatively little change until the growing animal attains approximately half its physiological maturity which then depend on the age at slaughter (Fiems et al., 2000). Overall, the effect of carcass fatness score or carcass fat weight on beef color traits, maybe explained by two reasons. First, the amount of fat content would play a role in the reflectance aspects of meat cuts (Hughes, Kearney, & Warner, 2014) due to both physicochemical aspects of fat and oxidation conditions that may occur during the ageing period. Second, the high amount of fat is already associated with a shift in muscle fibers known to be related with mitochondrial density, as endurance exercise stimulates mitochondrial biogenesis and increases mitochondrial capacity in response to β -oxidation, thereby playing pivotal role in meat color determinism (Astruc & Venien, 2017; Matarneh et al., 2017; Neethling et al., 2017).

3.4.5. pH24h

The pH24h (**Fig. 4**) affected a^* , C^* and h^* , which all reflect the redness/vividness or discoloration of meat. As pH24h increased, a^* and C^* decreased ($a^* \text{ Mag} = -3.08$; $P < 0.001$

and C^* Mag = -3.06 ; $P < 0.001$), and h^* increased (Mag = $+2.69$; $P < 0.01$). However, no significant impact was found on L^* and b^* coordinates ($1.23 < \text{Mag} < 1.35$; $P > 0.05$). The effect of pH on meat color was extensively studied (Abril et al., 2001; Neethling et al., 2017; Page et al., 2001). For example, (Abril et al., 2001) investigated the changes in color traits (L^* , a^* , b^* , C^* and h^*) of beef from two divergent pH classes (pH < 6.1 and pH ≥ 6.1). The authors found that pH had a significant effect on all the color variables. Furthermore, the biological mechanisms are to some extent well established today (Gagaoua et al., 2015; Hughes, Clarke, Purslow, & Warner, 2017; Hughes et al., 2014; Neethling et al., 2017). In line with our findings, (Page et al., 2001) and (Gagaoua, Couvreur, et al., 2017) reported negative relationship between a^* and pH, which would partially explain the greater a^* -values found in young bulls than cows cuts (**Table 2**). Similar findings were reported by (Węglarz, 2010). Moreover, after bleeding, *post-mortem* muscle pH decline is accompanied by myofibrillar structural changes that might modify the degree of light penetrating or scattering by the structural elements (Gagaoua et al., 2015; Gagaoua, Terlouw, et al., 2017; Hughes et al., 2014). Warner's research group, reported very recently the detailed mechanisms by which pH would affect the visual aspects of meat color using reflectance confocal laser scanning microscopy (Hughes et al., 2017). According to these authors, pH decline induces alterations in the arrangement of myofilaments, thereby expelling fluids that may affect the color aspects.

3.5. Principal component regression for meat color traits prediction and study of the overall relationships with the covariates

The overall relationships of the studied covariates with meat color traits were assessed by PCRs (**Fig. 5**), which is a principal component analysis (PCA) followed by multiple linear regression (Gagaoua et al., 2018). The PCRs highlighted **i**) the significant relationships ($P < 0.05$), **ii**) the prediction power of the models and **iii**) the direction of the impact (negative, positive or null) of the covariates in the models at the same time. For each of the models, a predictive accuracy is given by RMSE. For L^* model (**Fig. 5A**), the PCR gave a model with R^2 -value of 66% ($P < 0.0001$) by including 5 covariates from 8: age at slaughter, carcass fat weight, carcass fatness score and slaughter weight (all negatively) and conformation score (positively). For a^* , b^* and C^* models (**Fig. 5B-D**), the PCRs gave models with R^2 -values of 85% ($P < 0.0001$), 81% ($P < 0.0001$) and 87% ($P < 0.0001$), respectively. They all included 7 common covariates and with the same direction: age at slaughter, carcass fat weight, carcass fatness score and slaughter weight (all positively) and conformation score, pH24h and carcass muscle weight (all negatively). Finally, for h^* model (**Fig. 5E**), the PCR gave a model with

R²-value of 45% ($P < 0.01$) by including 2 covariates only from 8: pH24h (positively) and age at slaughter (negatively). These overall relationships as well as the ability of the covariates to fit into the models of the five color traits are summarized in **Fig. 5F**. The relationships comfort as well the results given above by the multivariate regression analysis (**Fig. 4**). The PCR results depict clearly, the non-association of hot carcass weight as discussed above with none of the color traits. The specificity and direction of the relationships are also summarized (**Fig. 5F**). These results showed that it is possible to achieve best models of color traits prediction, namely a^* , b^* and C^* , using all the variables related to animal, muscle carcass characteristics at the same time. These models, with easy use in slaughterhouses, highlight the potential of different production factors to predict color traits. In the future, we suggest using the same approach to study meat color stability on beef cuts. This analysis indicated that variables characterizing carcasses gave great and better information for prediction purposes compared to muscle composition as already reported in earlier studies some of which are cited above.

4. Conclusions

Different variables related to the rearing factors, animal, carcass and muscle characteristics affect initial fresh meat color traits measured 24h *post-mortem* on LT muscle of purebred Charolais animals. From the findings of this study, the following conclusions were made:

- Strong effects of year of birth, experiment, sex and their interactions were found for all the color traits (L^* , a^* , b^* , C^* and h^*).
- In agreement to our hypothesis, the base models after removing of the effects of rearing factors, revealed strong relationships with animal, muscle and carcass traits on each color trait.
- Age at slaughter, carcass fat weight, carcass fatness score and slaughter weight affected all positively a^* , b^* and C^* traits.
- In contrast to these, EUROP conformation score, pH24h and carcass muscle weight affected negatively a^* , b^* and C^* traits.
- From these findings, it seems that increased age lead to get intense color (low h^*) and darker and vivid meat (high a^* , b^* and C^*).
- Similarly, the selection of animals for high slaughter weight will have positive effect and lead to red and vivid meat but with negative impact on brightness.

- Carcass conformation and fat cover scores in beef cattle would be useful for not only carcass grading but also to act as indirect indicators of meat color evaluation and segregation between light and dark-red of Charolais carcasses.
- No significant effect of hot carcass weight was found with any of the color traits.
- Principal component regression (PCR) analyses validated the impact and direction of the studied variables on each color trait and allowed to check both their direction and impacts on the prediction models.
- The prediction PCR models on Charolais bulls, cows and steers explained more than 80% of the variability of a^* , b^* and C^* -values, while low prediction powers were found with L^* (66%) and h^* (45%).
- The use of the studied co-variables to predict and manage meat color traits would be of great importance for the stakeholders.

From the findings of this study, it seems that joint management of conformation and fat content of the carcasses in relation with age and final animal weight during the fattening period and its duration is a possible task to obtain the desirable and targeted beef color.

Conflict of interest

The authors declare that no competing interests exist.

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Figure captions

Fig 1. Schematic representation of the excision steps of the 6th rib from the right half of the carcass for dissection and estimation/prediction of carcass composition (muscle, fat and bone) using Robelin and Geay, (1975) prediction equations. The first step consist to saw the carcass between the 6th and 7th ribs to quarter the carcass then excise and remove the 6th rib for its dissection.

Fig 2. Principal component analysis (PCA) showing both the loading and score plots of L^* , a^* , b^* , C^* and h^* color coordinates for *Longissimus thoracis* muscle used to discriminate between the three sexes of Charolais cattle [young bulls (n = 446), steers (n = 223) and cows (n = 218)]. The projection of the individuals of young bulls (blue), steers (orange) and cows (green) are encircled in ellipses (x,y-means \pm x,y-standard deviation (SD)) using the corresponding schematic colors. The overall Kaiser-Mayer-Olkin score of the PCA was 0.86 (Bartlett's test of sphericity was significant, $P < 0.001$). The eigenvalues of PC 1 and PC2 are 3.3 and 1.1, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Fig 3. Principal component analysis (PCA) performed using color coordinates (L^* , a^* , b^* , C^* and h^*) of *Longissimus thoracis* muscle of Charolais breed and animal, muscle and carcass characteristics covariate data. The projections of the individuals for each sex are shown by their barycenter's \pm SD on both axes. The color coordinates are given in bold characters. The overall Kaiser-Mayer-Olkin score of the PCA was 0.75 (Bartlett's test of sphericity was significant, $P < 0.001$). The eigenvalues of PC 1 and PC2 are 5.1 and 2.9, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Fig 4. The effect of animal, muscle and carcass characteristics covariate data on L^* , a^* , b^* , C^* and h^* of *Longissimus thoracis* muscle of Charolais animals. The magnitude of effects representing the difference between the minimum and maximum of predicted L^* , a^* , b^* , C^* and h^* are given above each co-variable and corresponding color coordinate. The values that were significant ($P < 0.05$) are all shown in bold characters. For example, age at slaughter had the strongest effect on C^* with a magnitude of 5.61 and the lowest (weak) effect on h^* with a difference mean value between the minimum and maximum predicted of 1.55.

Fig 5. Principal component regression (PCR) performed for **A)** Lightness (L^*), **B)** Redness (a^*), **C)** Yellowness (b^*), **D)** Chroma (C^*) and **E)** Hue angle (h^*) color traits with animal, carcass and muscle characteristics. The regression coefficients for all the covariates are given from the highest and significant to the lowest and non-significant. Thus, green and red colors highlight the positive and negative coefficients in the PC regressions, respectively. The non-significant covariate were in blank. **F)** Summary of the retained covariates in the PCR to explain/predict each of the five color traits. RMSE: Root-Mean-Square Error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 1. Description of dependent variable (color parameters) and covariate data (animal, muscle and carcass characteristics) measured on *Longissimus thoracis* muscle of Charolais cattle from the three sexes.

	Number	Mean	SD	Range
Lightness (L^*)	887	33.17	5.96	13.24 – 48.04
Redness (a^*)	887	17.38	5.02	7.79 – 39.97
Yellowness (b^*)	887	17.29	4.37	8.02 – 33.33
Chroma (C^*)	887	24.59	6.37	14.11 – 49.13
Hue angle (h^*)	887	45.00	4.72	30.88 – 58.51
<i>Animal, muscle and carcass characteristics covariates</i>				
Age at slaughter (month)	887	35.8	26.6	14.3 – 107
Slaughter weight (kg)	887	689	80.7	424 – 926
EUROP conformation score (1 - 15) ^a	887	9.82	1.28	7.0 – 12
Carcass fatness score (1 – 5) ^b	887	2.94	0.76	2.0 – 4.0
Hot carcass weight (kg)	887	420	52.0	251 – 565
Carcass fat weight (kg) ^c	880	70.9	18.0	27.4 – 140
Carcass muscle weight (kg) ^c	880	289	37.6	178 – 408
pH24h	846	5.57	0.10	5.10 – 6.07

^a EUROP classification grid for carcass conformation scores from P- = 1 to E+ = 15.

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

^c Estimated from the 6th rib composition obtained by dissection. Fat weight is the sum of internal, subcutaneous and intermuscular fat weights.

Table 2. Variance analysis on meat color parameters, animal, muscle and carcass characteristics of Charolais cattle per sex.

Variables	Young bulls ^a (n = 446)	Steers (n = 223)	Cows (n = 218)	SEM ^b	P-value ^c
Beef color parameters					
Lightness (L^*)	35.95 ^a	32.35 ^b	28.49 ^c	0.200	***
Redness (a^*)	14.74 ^c	18.89 ^b	21.46 ^a	0.169	***
Yellowness (b^*)	15.35 ^c	18.70 ^b	20.10 ^a	0.147	***
Chroma (C^*)	21.36 ^c	26.64 ^b	29.45 ^a	0.214	***
Hue angle (h^*)	46.00 ^a	44.84 ^b	43.29 ^b	0.159	*
Animal and carcass characteristics covariates					
Age at slaughter (month)	16.97 ^c	29.18 ^b	81.12 ^a	0.893	***
Slaughter weight (kg)	662 ^b	722 ^a	715 ^a	2.711	***
EUROP conformation score (1 - 15)	10.74 ^a	9.07 ^b	8.72 ^c	0.043	***
Carcass fatness score (1 – 5)	2.48 ^c	3.39 ^b	3.50 ^a	0.025	***
Hot carcass weight (kg)	410 ^c	440 ^a	424 ^b	1.749	***
Carcass fat weight (kg)	62.18 ^c	79.16 ^b	82.16 ^a	0.606	***
Carcass muscle weight (kg)	296 ^a	291 ^a	279 ^b	1.267	**
pH24h	5.58 ^a	5.57 ^a	5.54 ^b	0.003	**

^a Least square means in the same row for gender effect not followed by a common letter (a-c) differ significantly: $P < 0.05$.

^b Standard error of mean

^c Significances : * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3. Values of fixed effects on unit difference in L^* , a^* , b^* , C^* and h^* of *Longissimus thoracis* muscle of Charolais animals. The minimum (Min), maximum (Max) and magnitude (Mag) between predicted means for Charolais steaks L^* , a^* , b^* , C^* and h^* by year of birth, experiment, sex and interaction.

	L^*			a^*			b^*			C^*			h^*		
	Min	Max	Mag _a	Min	Max	Mag	Min	Max	Mag	Min	Max	Mag	Min	Max	Mag
Year of birth	25.84	38.28	12.43_b	13.44	23.11	9.70	12.35	22.22	9.87	18.32	32.10	13.78	37.20	46.23	9.03
Experiment	29.11	34.21	5.10	14.77	20.96	6.19	14.38	18.08	3.70	24.41	28.48	4.07	42.62	46.87	4.25
Sex	28.48	35.82	7.33	14.70	21.46	6.76	15.35	20.10	4.75	21.33	29.45	8.12	43.29	46.07	2.78
Interaction (year of birth x experiment x sex)	24.98	43.90	18.92	12.70	24.69	11.99	12.07	22.91	10.84	17.61	33.51	15.90	36.11	51.59	15.48

^a Bold characters highlight the magnitude values that were significant ($P < 0.05$).

^b In the case of this row referring to year of birth effect on L^* , the difference between the minimum and maximum predicted mean (magnitude) is 12.43 that highlight strong effect ($P < 0.05$) of year of birth compared to experiment with a magnitude of 5.10. The magnitude is a value that would be taken in account if for example the concerned effect was studied, by adding the numerical value to the comparisons.

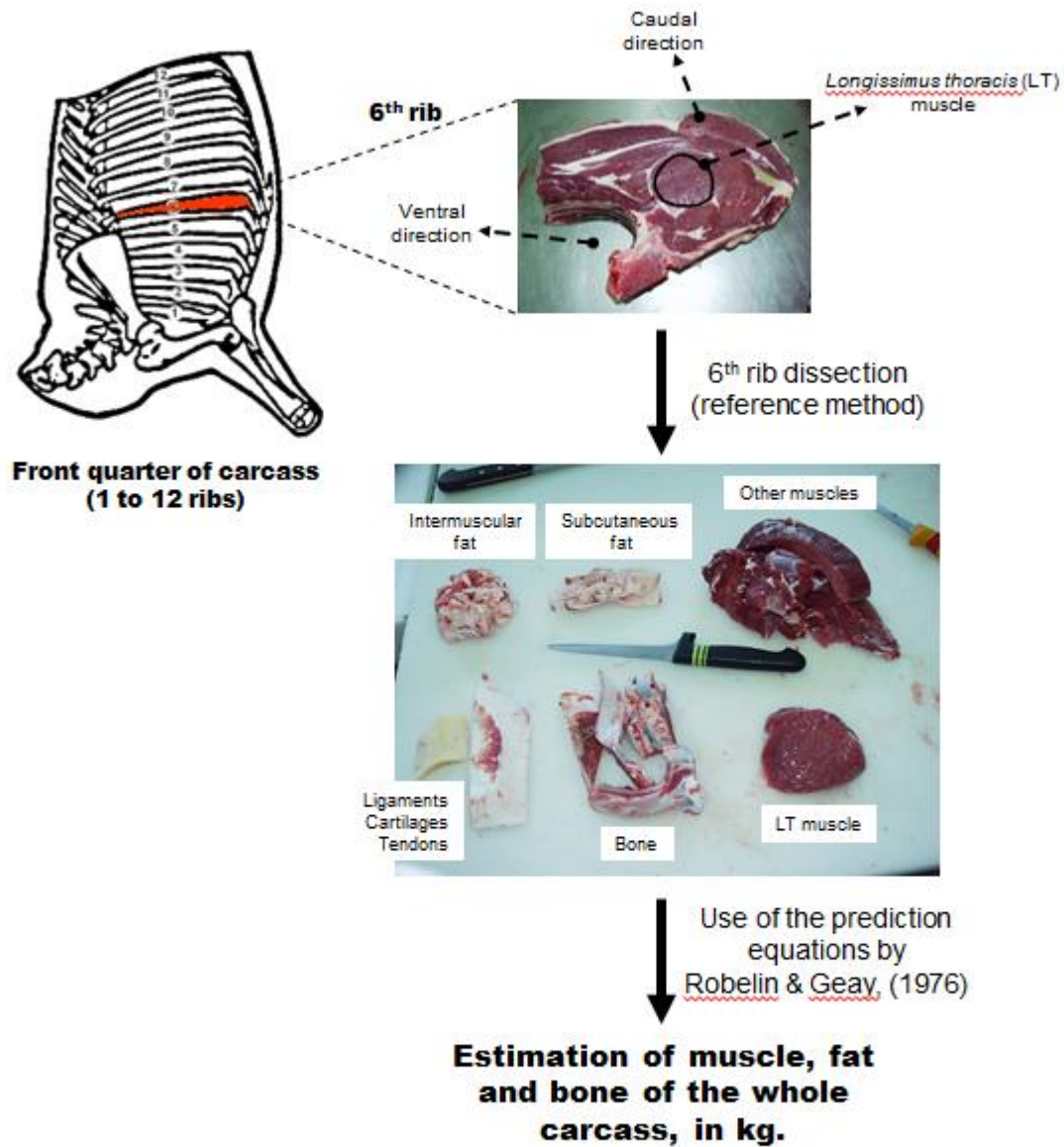


Fig 1.

AC

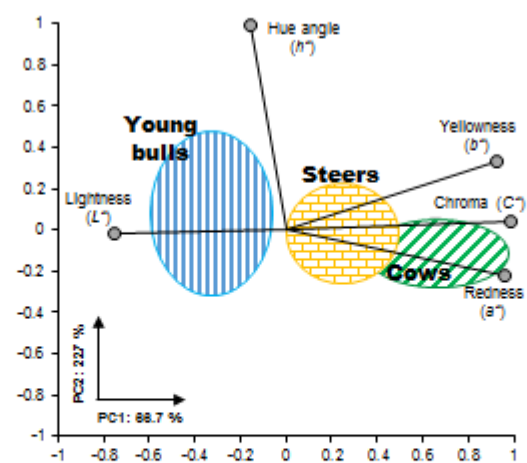


Fig 2.

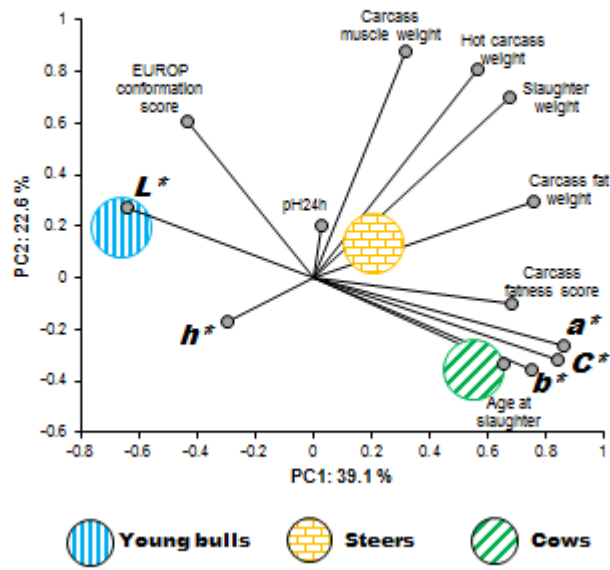


Fig 3.

ACCEPTED MANUSCRIPT

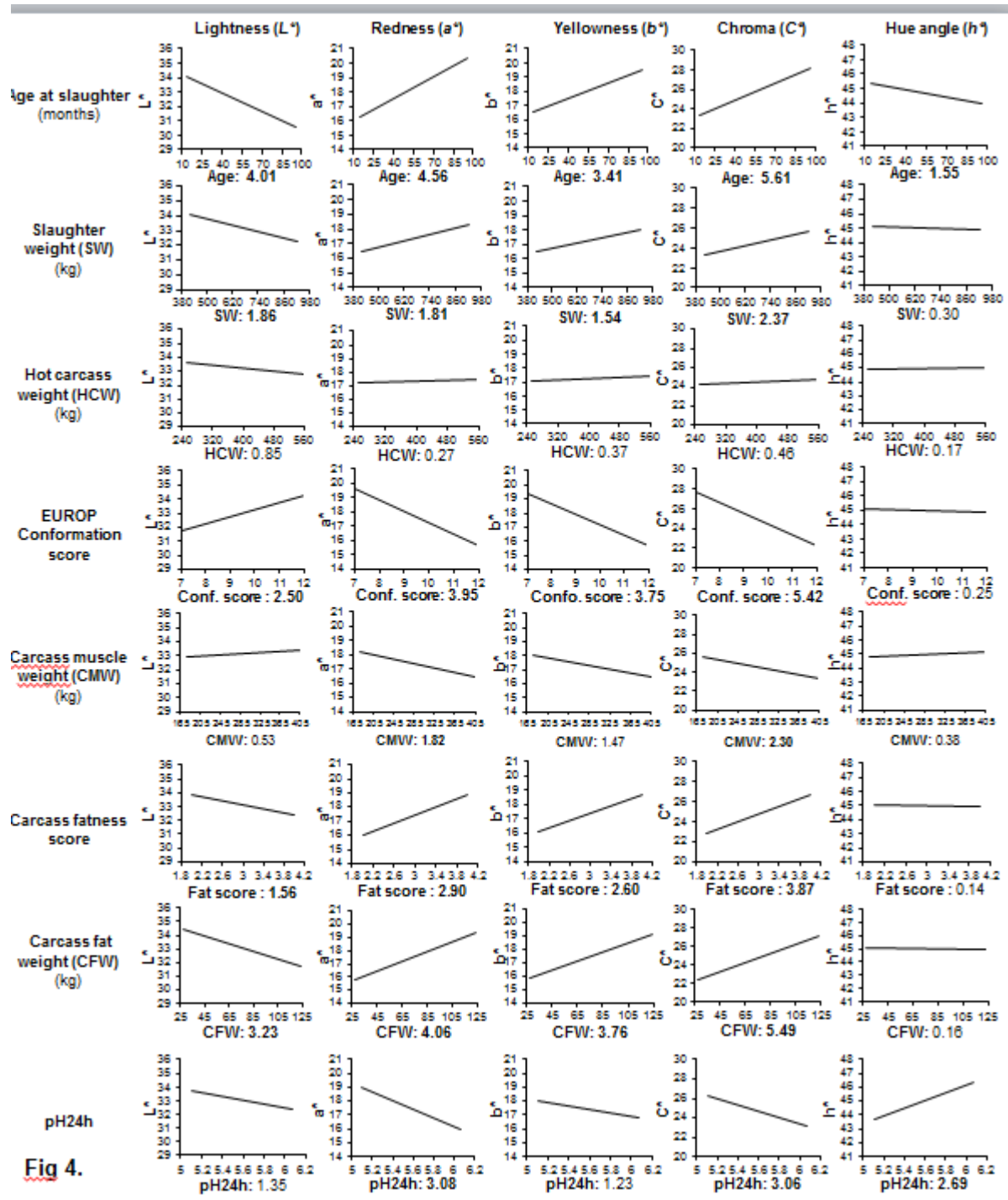
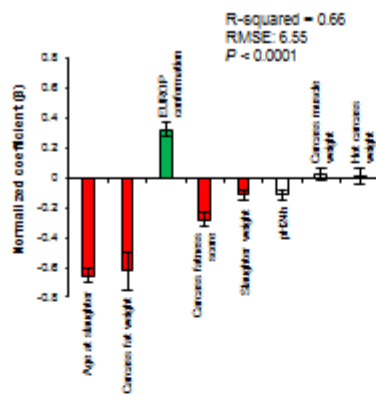
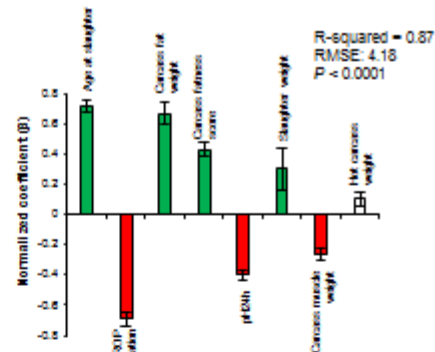
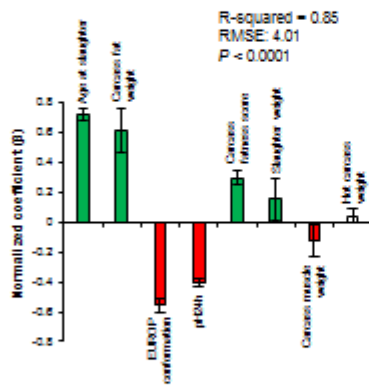
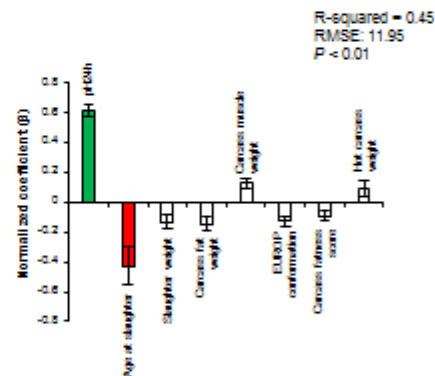
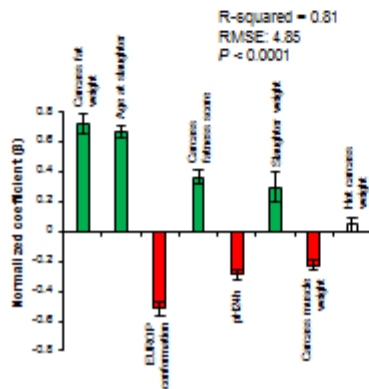


Fig 4.

A) Lightness (L^*)**D) Chroma (C^*)****B) Redness (a^*)****E) Hue angle (h^*)****C) Yellowness (b^*)****F) Overall relationships**

Animal, carcass and muscle characteristics covariates	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Chroma (C^*)	Hue angle (h^*)
Age at slaughter	Red	Green	Green	Green	Red
Slaughter weight	Red	Green	Green	Green	Green
EUROP conformation score	Red	Green	Green	Green	Green
Carcass fatness score	Red	Green	Green	Green	Green
Hot carcass weight	Red	Green	Green	Green	Green
Carcass fat weight	Red	Green	Green	Green	Green
Carcass muscle weight	Red	Green	Green	Green	Green
pH24h	Green	Red	Red	Red	Green

Fig 5.

Highlights

- Principal component analysis distinguished efficiently between Charolais breed sexes using $L^*a^*b^*$ color coordinates.
- Ultimate pH was greater than 5.8 for 1.8% of cattle, and affected a^* , C^* and h^* traits.
- Increased age lead to intense color (low h^* , -1.55 units) and darker and vivid meat.
- Fatness score and carcass fat weight are precursors of red and vivid Charolais beef.
- High degree of muscularity or slaughter weight, lead to lighter and darker meat, respectively
- Principal component regression (PCR), a promising tool for the prediction of beef color traits.