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A B S T R A C T

The lack of consumer feedback on beef eating quality contributes to reduced beef consumption in Europe. The Meat Standards Australia (MSA) grading scheme can assess the palatability of beef carcasses usually graded at the 10th *thoracic vertebrae*. However, the European beef industry relies on late-maturing breeds usually cut at the 5th *vertebrae* due to commercial reasons. Data from 55 young bulls and heifers of late-maturing breeds were collected in an Italian slaughterhouse following the MSA guidelines at both carcass grading sites and sides. Intramuscular fat levels were assessed through two scores and used with other variables to feed the MSA model, which predicts the MSA index, the meat-eating quality scores (MQ4) for 5 muscles and for each carcass grading site × side combination. The scores were analyzed using a mixed linear model. A correlation analysis was conducted to predict the variables measured at the 10th site using their correspondent at the 5th carcass grading site. A stepwise regression was conducted to understand the weight of each measured variable on marbling and MQ4 scores measured both at 5th and 10th carcass grading sites. Results showed significantly higher value for the studied traits at the 5th carcass grading site, while carcass side had no significant impact. The equations had high predictive capability and MSA marbling score played a key role in explaining the variability across carcass grading sites. The differences in marbling and MQ4 scores between the carcass grading sites suggest considering this factor if the MSA grading system will be applied to Europe.

1. Introduction

The decline in beef consumption is one of the major issues of the European beef industry which lacks a mechanism for delivering feedback from the consumer to the producer in terms of beef palatability (Bonny et al., 2018a; Hocquette et al., 2018). In addition, evolution in the consumer demands, and the competition with emerging and alternative sources of protein are influencing the beef market (Bonny, Gardner, Pethick, & Hocquette, 2015; De Marchi, Costa, Pozza, Goi, & Manuelian, 2021). Thus, the European beef production system needs to be more competitive and consumer-focused (Bonny et al., 2018a). Indeed, the European beef grading system is based on evaluations that reflect carcass features, such as carcass weight, sex, conformation (EUROP grading system), and external carcass fat (EUROP fat score) (Liu et al., 2020a; Monteils et al., 2017). Unfortunately, this grading scheme rewards carcass weight and yield rather than the beef sensory traits including product homogeneity and consumers' satisfaction (Hocquette et al., 2018; Liu et al., 2020a; Polkinghorne, Philpott, Gee, Doljanin, & Innes, 2008a). One of the most advanced beef grading schemes is the Meat Standards Australia (MSA), which predicts beef palatability of several cuts as it interacts with cooking method (Hocquette et al., 2017). The MSA grading scheme predicts the beef eating quality using *pre*-slaughter features and grading information collected before and at the slaughterhouse facility (Bonny et al., 2018b). In particular, the animals must follow on-farm requirements to be evaluated with the MSA methodology and the grading scheme is applied by a trained and

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accredited grader in part on the *Longissimus thoracis et lumborum* (LTL) after carcass processing at any ribbing site between the 5th and the 13th *vertebrae* (Polkinghorne & Thompson, 2010). The slaughter information is used to feed a multiple regression model (MSA model) to predict a single palatability score (MQ4 score) for each "muscle × cooking method" combination. The model also predicts the MSA index, which describes the average consumer eating experience for the whole carcass combining all MQ4 scores from each muscle based on their most common cooking method, weighted by the proportions of the individual cut relative to total weight of all cuts (Bonny et al., 2018a). The MSA index is usually calculated for a theoretical ageing time of 5 days as indicated by McGilchrist, Polkinghorne, Ball, and Thompson (2019), whereas the MQ4 can be calculated assuming different ageing times. Among the grading traits, marbling is one of the most related to sensory quality of the meat and MSA grading scheme estimates it as a proxy of intramuscular fat content. In fact, several studies have shown that intramuscular fat content is positively correlated with tenderness, juiciness, flavor, and overall liking (Meat Standard Australia – AUS-MEAT, 2020; Hocquette et al., 2011; Thompson, 2004; Dransfield et al., 2003; Thompson, 2001). Intramuscular fat is the result of adipocyte hyperplasia and hypertrophy (Hocquette, Legrand, Jurie, Pethick, & Micol, 2010; Jo et al., 2009) and it appears as white flecks within muscle fibers. The intramuscular fat deposition decreases moving from the cranial to the caudal part of the animal, and overall, it increases with age(Meat Standards Australia - AUS-MEAT, 2018). As discussed by Monteils et al. (2017), including marbling and other intrinsic quality traits in the European grading scheme could enhance the quality and the economic value of beef products.

In the last years, many studies have been conducted in European countries to study the MSA methodology in the EU context. In almost all cases, results were promising (Bonny et al., 2016a; Hocquette et al., 2011; Legrand, Hocquette, Polkinghorne, & Pethick, 2013; Liu et al., 2020a) so that the International Meat Research 3G Foundation was launched to implement mainly in European countries a grading scheme like the MSA system (Hocquette et al., 2020). The MSA grading system was developed in Australia, where the most reared categories of cattle are young steers and heifers of early maturing breeds. In addition, carcasses are generally graded on cut surface of *LTL* from the 10th to 12th *thoracic vertebrae*, meanwhile in Europe the most reared animals are bulls, heifers, and cull cows of late-maturing breeds and their carcasses are mainly cut at the 5th *vertebrae* for commercial reasons. To our knowledge, a very limited number of studies have investigated the differences on MSA grading system between the 5th and 10th *thoracic vertebrae* considering European cattle breeds. Liu et al. (2021) have investigated this aspect with French Limousine cull cows and reported no significant difference in marbling scores between the 5th and 10th carcass grading sites. However, no information is available for still growing animals such as young bulls and heifers of late-maturing breeds (e.g., Charolais, Limousine, French crosses), that represent a major part of the European beef production system as well as of the Italian beef supply chain (De Roest, 2015). Moreover, considering that young bulls and heifers from the late-maturing European breeds will have a greater propensity to develop muscle rather than fat, the homogeneity of intramuscular fat distribution between the 5th and 10th grading site requires further investigation. Therefore, the aim of the current study was to investigate the impact of grading site (5th vs. 10th) and carcass side (left vs. right) on marbling scores, MQ4 scores, and MSA index in young bulls and heifers of late-maturing breeds.

2. Material and methods

2.1. Animal description and experimental design

The data used in this study included 55 carcasses (29 young heifers and 26 young bulls) processed in a commercial slaughterhouse (Cittadella, Italy) from April 2022 to August 2022. Animals were of Charolais breed (18 females and 4 males) and French crosses (11 females and 22 males). Detailed information about the specific breeds that generated the crossbred animals was not available. The animals represented beef breeds typically reared in the North-East of Italy, which are mostly imported from France and reared under intensive conditions in Italian specialized fattening farms (Santinello et al., 2022). Animals were fed a diet rich in concentrates (Supplementary Table 1) to reach a slaughter weight of about 750 kg after six months of fattening (Gallo, De Marchi, & Bittante, 2014).

Carcasses were evaluated after being equally divided into two halves and *post-mortem* Achilles hung for 24 h. Carcasses were graded by a certified grader under the auspices of AUS-MEAT and the International Meat Research 3G Foundation, who assessed ossification, and hump height following the Australian beef chiller assessment system standards (ABCAS; Meat Standards Australia - AUS-MEAT, 2018). To reduce possible subjectivity and assure a blind assessment, the MSA chiller assessor graded marbling, total rib fat and eye muscle area at the 5th and 10th *thoracic vertebrae* sites using a steak (6 cm height) removed from each carcass half (right and left) and positioning on a table with the same lighting conditions (Supplementary Fig. 1). Steaks were graded at least 20 min after the cutting to allow meat blooming. Thus, for marbling, rib fat depth, eye muscle area and ultimate pH each animal had four measurements: right carcass - 10th site, right carcass - 5th site, left carcass - 10th site, and left carcass - 5th site.

2.2. Data specification and grading scheme

According to the ABCAS standards, ossification score was measured by visual assessment of calcification degree in the *sacral, lumbar, and thoracic vertebrae* (Meat Standards Australia - AUS-MEAT, 2018). Carcass hump height (mm) was measured from the most dorsal point of the hump to the dorsal edge of the *ligamentum nuchae*. To assess marbling, two scores measured by visual assessment of *LTL* muscle were used: the AUS-MEAT marbling and the MSA marbling. The first describes the amount of intramuscular fat ranging from 0 to 9 in increments of 1 and the second ranges from 100 to 1190 in increments of 10, and describes the amount, the size, the fineness, and the distribution of intramuscular fat inclusion in the muscle. The MSA marbling is based on the United States Department of Agriculture system (United States Department of Agronomy - USDA, 2017) and provides a more precise scale for marbling if compared with the AUS-MEAT marbling. The rib fat depth is the measure of subcutaneous fat (mm) along the rib eye muscle. The European grading score information (EUROP conformation and fat score) and animals' characteristics are reported in Table 1.

2.3. Prediction of MQ4 scores and MSA index

The traits collected at the slaughterhouse were used to predict MQ4 scores for each muscle through the MSA model which uses a multiple regression approach to predict quality scores of individual muscles for a range of animals' categories, ageing time, hanging method, and cooking methods. There are different versions of the model because it is still improving in terms of predictability through the addition of other parameters to the multiple regression approach, such as new classes of cattle categories and cooking methods (Bonny et al., 2018b). In this study, the SP2009 version of the MSA model was used to predict the MQ4 scores and MSA index based on the assumption that the accuracy of the prediction was at least as good as previously observed in Europe (Bonny et al., 2016b). The SP2009 model includes, among other traits, animal sex, carcass weight (kg), hanging method, hump height (mm), ossification score, MSA marbling score, rib fat depth (mm), ultimate pH, and days of ageing (Bonny et al., 2018a). The predictions of the MQ4 scores were obtained for 4 muscles (CUB045 - *Longissimus thoracis*; STA045 - *Longissimus thoracis et lumborum, anterior striploin piece*; RMP131 - *Gluteus medius*; TFL052 - *Obliquus internus abdominis)* according to the most common cooking method at 10 days of ageing:

Table 1
Descriptive statistics of animal and carcass traits of Charolais (18 females and 4 males) and French crosses (11 females and 22 males) cattle.

Trait	Mean	CV (%)	Minimum	Maximum
Animal characteristics				
Slaughter age (days)	575.9	20.57	413	947
Average slaughter live weight (kg)	639.4	87.10	485	881
Length of the fattening cycle (days)	231.7	77.43	187	537
Average daily carcass gain (kg/day)	0.69	0.10	0.52	1.00
Post-mortem				
Dressing percentage (%)	57.19	2.55	51.06	61.00
Hot carcass weight (kg)	363.5	57.41	248	516
EU Conformation score[1]	11.71 (U+)	1.40	8.00	14.00
EU Fat score[2]	7.07 (3-)	1.39	5.00	8.00

[1] European conformation score were converted from P (−/=/+), O (−/=/+), R (−/=/+), U (−/=/+), and E (−/=/+) to classes from 1 (P-) to 15 (E+) following Hickey et al. (2007).

[2] European fat scores were converted from 1 (−/=/+), 2 (−/=/+), 3 (−/=/+), 4 (−/=/+), and 5 (−/=/+) to classes from 1 (1-) to 15 (5+) following Hickey et al. (2007).

Table 2
Descriptive statistics of Meat Standards Australia (MSA) traits and MSA predicted meat quality scores (MQ4) calculated as an average of all the measurement made both at the 5th and 10th carcass grading sites, and right and left grading sides.

Trait	Mean	CV (%)	Minimum	Maximum
Meat Standards Australia				
AUS-Meat marbling	2.11	1.16	0.00	7.00
MSA marbling	458	110	280	940
Ossification score	175	18.5	130	230
Eye muscle area (cm^2)	96.4	12.8	67.0	153
Total rib fat depth (mm)	10.4	4.94	1.00	23.0
Rib fat depth (mm)	6.05	3.52	1.00	17.0
Hump height (mm)	63.8	24.0	5.00	130
Cut predicted MQ4 scores[1]				
CUB045 GRL	67.6	4.01	61.0	77.0
STA045 GRL	62.2	5.32	53.0	74.0
RMP131 GRL	54.2	3.35	51.0	58.0
RMP131 RST	62.7	2.92	59.0	67.0
TFL052 SF	72.3	4.01	65.0	83.0
MSA index 5 days[2]	61.0	3.21	56.0	67.0

[1] Predicted MQ4 was calculated for five "cut × cooking method" combinations. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 10 days and cooked according to the most common cooking method for each cut; CUB045 GRL = MQ4 score of CUB045 (*Longissimus thoracis*) assuming the grilling cooking method; STA045 GRL = MQ4 score of STA045 (*Longissimus thoracis et Lumborum*, anterior striploin piece) assuming the grilling cooking method; RMP131 GRL = MQ4 score of RMP131 (*Gluteus medius*) assuming the grilling cooking method; RMP131 RST = MQ4 score of RMP131 assuming the roasting cooking method; TFL052 SF = MQ4 score of TFL052 (*Obliquus internus abdominis*) assuming stir-frying cooking method.

[2] MSA index 5 days = carcass predicted MSA score calculated as the weighed sum of the predicted MQ4 scores of all MSA cuts. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 5 days and cooked according to the most common cooking method for each cut.

grilling method (GRL) for CUB045, STA045, and RMP131, and stir-frying method (SF) for TFL052. For RMP131, MQ4 score was calculated also for roasting method (RST). The prediction of MQ4 scores was made 4 times for each "cut × cooking method" combination with the same input except for marbling score which was measured both at the 5th and 10th grading sites on left and right sides of the carcasses. The ageing time of 10 days was assumed because it is the most common ageing time used by the Italian slaughterhouse retailers.

Moreover, the model provided also the MSA index which is the weighed sum of the predicted MQ4 scores of all MSA cuts as reported above. The MSA index was developed to provide feedback to the producer about the potential eating quality of beef carcasses with the possibility to rank the animals and monitor the impact of management and genetic changes on the eating quality (McGilchrist et al., 2019). The MSA index (MSA index 5 days) was calculated four times assuming the standard ageing time of 5 days with the same input except for marbling score which was measured both at the 5th and 10th grading sites on left and right carcasses. More detailed information about the MSA methodology has been reported by Bonny et al. (2018b), McGilchrist et al. (2019), and Polkinghorne, Thompson, Watson, Gee and Porter (2008b).

2.4. Statistical analysis

The dataset was analyzed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Before calculating the descriptive statistics, the EUROP conformation and fat scores were converted into a continuous 15-point scale as described in Hickey, Keane, Kenny, Cromie, and Veerkamp (2007). A mixed linear model was performed using AUS-MEAT marbling, MSA marbling, MQ4 scores, and MSA index 5 days using the MIXED procedure of SAS:

$$y_{ijklmn} = \mu + \text{sex}_i + \text{breed}_j + \text{side}_k + \text{site}_l + (\text{side} \times \text{site})_{kl} + \text{slaughter date}_m + \text{animal}_n + e_{ijklmn}, \quad (1)$$

where y_{ijklmn} is the dependent variable; μ is the overall mean; sex_i is the fixed effect of the ith sex of the animal (i = male or female); breed_j is the fixed effect of the jth breed of the animal (j = Charolais or crossbred); side_k is the fixed effect of the kth side of the carcass (k = left or right); site_l is the fixed effect of the lth carcass grading site (l = 5th or 10th grading site); $(\text{side} \times \text{site})_{kl}$ is the fixed interaction effect between side and site; slaughter date_m is the random effect of the mth slaughter date (m = April 2022 to August 2022); animal_n is the random effect of the nth animal (n = 1 to 55) $\sim N(0,\sigma_a^2)$, where σ_a^2 is the animal variance; and e_{ijklmn} is the random residual $\sim N(0,\sigma_e^2)$, where σ_e^2 is the error variance. Data are presented as least squares means and standard error, and a multiple comparison of least squares means was performed using Bonferroni *post-hoc* test ($P < 0.05$). To determine the regression parameters between the MSA marbling score, AUS-MEAT marbling score, MSA index 5 days, and MQ4 scores for each "muscle × cooking method" combination measured at both carcass sites (5*th* and 10*th* ribs), a regression analysis was performed on raw data (Supplementary Fig. 2). Moreover, a stepwise multiple regression analysis was performed for MSA index 5 days and MQ4 scores for each "muscle × cooking method" combination to determine how the explained variance changes while adding more factors.

3. Results and discussion

3.1. Variability of MSA beef quality traits and MQ4 predictions

The descriptive statistics of overall MSA traits, predicted MQ4 scores, and MSA index 5 days measured or predicted both at the 5th and 10th carcass grading site and left and right carcass side are shown in Table 2.

On average, the MSA marbling score was 458 with the greatest coefficient of variation (110%) compared to other traits that were characterized by lower variability (from 1.16% to 23.99% for AUS-Meat marbling and hump height, respectively).

The average MSA marbling score obtained in our study was greater than the values of 293 to 329 reported by Liu et al. (2020b) derived from a diverse range of European cattle. Carcasses of the current study were heavier than those of Liu et al. (2020b) which is consistent with higher expression of marbling. Moreover, total rib fat depth and subcutaneous rib fat depth values were greater than those observed by Liu et al. (2023) for young Angus × Salers crossbreeds reared in a pasture-based system. Considering that we did not have specific information about management, it is not possible to explain the differences between values of MSA marbling score of the current study with those retrieved from the literature. Nevertheless, the Italian management system is characterized by high levels of concentrate (e.g., grain, maize silage) in the diet which results in a high energy finishing feed practice compared to a typical pasture system (Duckett, Neel, Fontenot, & Clapham, 2009) which will encourage higher fat and higher marbling deposition.

Concerning the predicted MQ4 scores (Table 2), the average values ranged from 54 to 72 for RMP131 GRL and TFL052 SF, respectively. Considering the possible range of variation of the MQ4 scores (0–100%), we observed animals with moderate values for MQ4 scores according to the investigated muscles and cooking methods (on average 61), and for all the MQ4 scores the coefficient of variation was very low (between 2.9 and 5.3%). The low variation for MQ4 score was likely due to the homogeneity of the animals through the Italian beef supply chain and the limited number of studied carcasses. According to the benchmarks reported by Polkinghorne et al. (2008b) the boundaries of 46, 64, and 77 correspond to 2/3-, 3/4-, and 4/5-thresholds of the indicated star grades; thus, meat from the animals of our study was mostly of medium quality (between 3 and 4 stars). In the Australian system these boundaries in combination with absolute MQ4 scores are used to sort cuts into eating quality classes that are sold under company brands and priced accordingly to quality grades (Pethick, Hocquette, Scollan, & Dunshea, 2021). Meanwhile, according to Legrand et al. (2013) who conducted a study to predict eating quality scores based on French consumers, the boundaries between 2/3, 3/4, and 4/5 star were 28, 61, and 80, respectively. Thus, the different boundaries could change according to the consumers involved and may affect the boundaries between star grades. The MQ4 scores of the present study were slightly greater than the values reported by Liu et al. (2020b) and this is partially explained by the heavier carcass and higher amount of marbling of the animals. In fact, the MSA marbling score does play a considerable role in the determination of the MQ4 scores and MSA index 5 days, especially when other predictive factors are held constant (hang, *Bos indicus* content, hormone growth promotion status) (Pethick et al., 2021). Moreover, it should be considered that the MSA model has been developed in the Australia beef industry (e.g., castrated males and heifers of early-maturing breeds) and thus, despite promising results, it may provide only a rough estimation of European carcasses quality because the Australian prediction models may not reflect accurately the influence of muscles, marbling score, and ultimate pH on eating quality within the European beef categories (Bonny et al., 2016b). The not-for-profit International Meat Research 3G Foundation (https://imr3gfoundation.org/) was launched to rebuild an equivalent of the MSA model called 3G model based on the same principles but by using European data from animals, carcasses, and consumer testing (Hocquette et al., 2020).

3.2. Effect of grading site on beef quality traits and MQ4 predictions

The effects of carcass grading side and site on marbling score, predicted MQ4 score, and predicted MSA index 5 days are reported in Table 3. The carcass grading side (left and right) did not significantly affect the marbling score, predicted MQ4 scores, and predicted MSA index 5 days, while the carcass grading site (5th and 10th) had a significant effect for all the traits ($P < 0.001$). The MSA marbling score, AUS-Meat marbling, MSA index 5 days, and all the MQ4 scores were higher at the 5th carcass grading site compared to the 10th. The interaction between the carcass grading side and carcass grading site was not significant for all the evaluated traits (data not shown). The overall results clearly underlined the different intramuscular fat deposition between the anterior and more posterior section of *Longissimus thoracis*; the hypothesis that intramuscular fat started to deposit from the anterior to the posterior part was reported in previous reports by Meat Standards Australia - AUS-MEAT (2018) and Taylor and Johnson (1992). Consequently, the higher level of marbling found at the 5th carcass grading site influenced all the MQ4 scores and MSA indexes which were significantly higher at the 5th compared to the 10th site. A possible explanation could be the anatomical differences between grading sites. Indeed, Schulz and Sundrum (2019) reported challenges in rib-eye identification and segmentation when using the VBG2000 system (Oranienburg, Germany), which was calibrated to grade at the 12th–13th rib. Furthermore, pictorial cards used to assess MSA traits were developed for the 10th–13th ribs, and thus this could have interfered with marbling visualization during grading session. Due to differences in size and anatomy of the rib-eye area at the 5th and 10th sites, images included portions of the *M. spinalis*, thereby impacting eye

Table 3
Least squares means and standard error of the mean (SEM) of Meat Standards Australia (MSA) traits and MSA predicted meat quality scores (MQ4) for carcass grading sides (Left and Right) and carcass grading sites (5th and 10th rib) effects.

Trait	Side		SEM	*P*-value	Site		SEM	*P*-value
	Left	Right			5th	10th		
Meat Standards Australia								
AUS-Meat marbling	2.02	1.94	0.17	0.144	2.24	1.72	0.17	<0.001
MSA marbling	449	444	16.0	0.211	472	421	16.0	<0.001
Cut predicted MQ4 scores[1]								
CUB045 GRL	67.5	67.5	0.45	0.798	68.1	66.8	0.45	<0.001
STA045 GRL	62.0	61.9	0.55	0.822	62.7	61.2	0.55	<0.001
RMP131 GRL	54.2	54.3	0.27	0.658	54.5	54.0	0.27	<0.001
RMP131 RST	62.8	62.8	0.28	0.420	63.0	62.6	0.28	<0.001
TFL052 SF	72.1	72.1	0.48	0.672	72.7	71.5	0.48	<0.001
MSA index 5 days[2]	61.0	61.0	0.34	0.944	61.4	60.6	0.34	<0.001

[1] Predicted MQ4 for five "cut × cooking method" combinations. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 10 days and cooked according to the most common cooking method for each cut; CUB045 GRL = MQ4 score of CUB045 (*Longissimus thoracis*) assuming the grilling cooking method; STA045 GRL = MQ4 score of STA045 (*Longissimus thoracis et Lumborum*, anterior striploin piece) assuming the grilling cooking method; RMP131 GRL = MQ4 score of RMP131 (*Gluteus medius*) assuming the grilling cooking method; RMP131 RST = MQ4 score of RMP131 assuming the roasting cooking method; TFL052 SF = MQ4 score of TFL052 (*Obliquus internus abdominis*) assuming stir-frying cooking method.

[2] MSA index 5 days = carcass predicted MSA score calculated as the weighed sum of the predicted MQ4 scores of all MSA cuts. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 5 days and cooked according to the most common cooking method for each cut.

muscle area determination and thus marbling prediction. The different size and muscle inclusion according to grading site could have even influenced marbling assessment. This study did not measure eye muscle area for all observations, and this prevented us to consider it as a response variable in the model to determine any differences between grading sites.

This suggests that the MSA model should take into consideration the intrinsic differences of marbling formation and deposition for the late-maturing breeds which are not usually reared in Australia. Contrary to our results, Liu et al. (2021) reported no difference between marbling deposition on the 5th and 10th carcass grading site in Limousine cull cows (with on average 9 years of age). This might be explained by the fact that Limousine cull cows are generally slaughtered when they have completed their growing phase, exhibiting a homogeneous marbling deposition along the grading sites, and this is likely to be related to the older age (Liu et al., 2021). Indeed, the cull cows are likely synthesizing less muscle compared to younger animals. However, young animals in the finishing Italian system are fed a diet rich in concentrates, which favors marbling deposition. Since marbling is the ratio between intramuscular fat tissue and muscle, this has probably increased the ratio of marbling in our animals. In support to our result, Acheson, Woerner, Walenciak, Colle, and Bass (2018) found that the marbling score collected by the official graders decreased from the 5th to the 13th carcass grading site and the magnitude of decreasing was 60 MSA marbling scores units.

Since we did not find any significant difference due to the grading on either left or right carcass sides, our results can empower the reliability and objectivity of the MSA chiller assessor who conducted the MSA evaluation. The chiller assessor was blinded with respect to the aim of the trial and hence was not influenced in attributing a value to both carcass grading sides and sites (Supplementary Fig. 1). The literature reports that there could be a certain bias related to the subjectivity of the evaluation according to the different chiller assessors (Moore et al., 2010). Many attempts to reduce this subjectivity have been conducted in the past years, such as using the measurement of chemical intramuscular fat presence and composition, the application of image analysis, ultrasound and near infrared technology (Konarska et al., 2013; Konarska, Kuchida, Tarr, & Polkinghorne, 2017; Stewart et al., 2021). In the study of Stewart et al. (2021), where MSA marbling score, AUS-MEAT marbling score, and chemical intramuscular fat were predicted using a prototype vision system, the authors reported a coefficient of determination (R^2) of 0.76, 0.70, and 0.78 between observed and predicted values, respectively. Meanwhile, the use of near-infrared spectroscopy can correctly predict the sensory quality of beef meat even if with low accuracy (Liu et al., 2003; Prieto, Roehe, Lavín, Batten, & Andrés, 2009). Kombolo-Ngah et al. (2023) reported that MSA marbling score can be predicted with low to moderate accuracy even for animals that were reared and slaughtered in different countries. However, the ability of these technologies to predict marbling score in the muscle is limited because they use two-dimensional approach to predict a three-dimensional trait such as marbling (Stewart et al., 2021). The three-dimensional nature of marbling (which slightly varies within the muscle in all directions) may be biased when predicted or expressed with two-dimensional technique that assesses marbling on a surface only (Kruk, Pitchford, Siebert, Deland & Bottema, 2012) Moreover, although previous studies have tried to predict marbling using different technologies, they have been generally structured around a single dataset and a relatively small population of animals which could have affected the indicators of the prediction (R^2 and Root Mean Square Error - RMSE) (Ferguson, Thompson & Cabassi, 1995). Vote et al. (2009) reported that image analysis on different grading sites can affect the predictions. Thus, it is important to develop or empower a more objective marbling evaluation according to the possible differences related to several factors including the grading site. The Australian AUS-MEAT system allows to use devices which produce objective evaluations only after a third part system check to assure the level of accuracy.

3.3. Effect of sex and breed on beef quality traits and MQ4 predictions

Supplementary Table 2 shows the effects of sex and breed on MSA and AUS-Meat marbling scores and MQ4 predicted values. Sex effect was not significant in explaining the variability for all the traits, except for RMP131 GRL and RST ($P < 0.05$). Usually, female animals deposit more intramuscular fat compared to entire males and thus we expected to find a difference (Schumacher, Del Curto-Wyffels, Thomson, & Boles, 2022). Probably, we failed to find a significant difference for the relatively low number of animals involved in the study. Concerning the breed effect, crossbred animals had higher AUS-Meat and MSA marbling compared to Charolais, but this did not significantly increase MQ4 scores and MSA index. Similar levels of marbling were reported by Liu et al. (2023) for French crossbred cattle (Angus x Salers). However, we did not know exactly the sire and the dam that generated crossbreds, and thus we did not have the elements to discuss this aspect further.

3.4. Prediction of MQ4 meat quality scores

Predictive regression models developed for each single trait at the 10th carcass grading site from the same scores at the 5th site are summarized in Table 4. All the equations showed high R^2 (0.77 to 0.92) for marbling, MQ4 predictions for 5 cuts, and the MSA index 5 days, confirming a high correlation between the traits measured at the two different carcass grading sites. Thus, good prediction of traits at the 10th carcass grading site can be achieved using traits measured at the 5th site, and this has relevant practical implications for European beef industry perspective since, for commercial reasons, the carcasses are usually cut at the 5th *thoracic vertebrae* (Liu et al., 2021). Moreover, our results agree with those of Schulz and Sundrum (2019) who observed that marbling scores collected at different carcass grading sites were highly correlated.

Tables 5 and 6 report the regression analysis to predict Meat Standards Australia meat quality scores (MQ4) of different muscles and cooking methods using rib fat, animal age and ossification plus the

Table 4
Regression models to predict Meat Standards Australia (MSA) marbling score and MSA meat quality scores (MQ4) at the 10th carcass grading site using the same traits recorded at the 5th carcass grading site as predictors (x).

Trait	Regression model
Meat Standards Australia	
AUS-Meat marbling	$0.9784^{***}(x) + 0.5582$ ($R^2 = 0.77$; RSD = 1.18)
MSA marbling	$1.0605^{***}(x) + 24.848$ ($R^2 = 0.89$; RSD = 114.18)
Predicted MQ4[1]	
CUB045 GRL	$0.9869^{***}(x) + 2.1597$ ($R^2 = 0.88$; RSD = 1.61)
STA045 GRL	$0.9828^{***}(x) + 2.5357$ ($R^2 = 0.89$; RSD = 1.49)
RMP131 GRL	$0.9815^{***}(x) + 1.4277$ ($R^2 = 0.92$; RSD = 1.01)
RMP131 RST	$0.9613^{***}(x) + 2.8727$ ($R^2 = 0.90$; RSD = 1.06)
TFL052 stir-fry	$0.9805^{***}(x) + 2.6396$ ($R^2 = 0.87$; RSD = 1.03)
MSA index 5 days[2]	$1.0013^{***}(x) + 0.6810$ ($R^2 = 0.92$; RSD = 1.63)

RSD = residual standard deviation.
[1] Predicted MQ4 for five "cut × cooking method" combinations. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 10 days and cooked according to the most common cooking method for each cut; CUB045 GRL = MQ4 score of CUB045 (*Longissimus thoracis*) assuming the grilling cooking method; STA045 GRL = MQ4 score of STA045 (*Longissimus thoracis et Lumborum*, anterior striploin piece) assuming the grilling cooking method; RMP131 GRL = MQ4 score of RMP131 (*Gluteus medius*) assuming the grilling cooking method; RMP131 RST = MQ4 score of RMP131 assuming the roasting cooking method; TFL052 SF = MQ4 score of TFL052 (*Obliquus internus abdominis*) assuming stir-frying cooking method.
[2] MSA index 5 days = carcass predicted MSA score calculated as the weighed sum of the predicted MQ4 scores of all MSA cuts. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 5 days and cooked according to the most common cooking method for each cut.
*** The asterisks indicate that the predictor is highly significant ($P < 0.001$).

Table 5
Stepwise regression analysis to predict Meat Standards Australia (MSA) meat quality scores (MQ4)[1] of different muscles and cooking methods on the basis of MSA traits recorded at the 5th carcass grading site.

MQ4	Equation[a]	R[2]
CUB045 GRL	0.020*(MB) + 58.633 (RSD = 1.45)	0.70
	0.021*(MB) - 0.004*(SA) + 60.123 (RSD = 1.39)	0.73
	0.022*(MB) - 0.004*(SA) - 0.023*(HUMP) + 61.517 (RSD = 1.22)	0.79
	0.021*(MB) - 0.0004(SA) - 0.020*(HUMP) - 0.037*(OSS) + 66.206 (RSD = 1.08)	0.84
	0.021*(MB) + 0.001(SA) - 0.011*(HUMP) - 0.035*(OSS) + 0.158*(RB) + 63.608 (RSD = 0.98)	0.87
STA045 GRL	0.024*(MB) + 51.196 (RSD = 1.79)	0.69
	0.026*(MB) - 0.004*(SA) + 52.795 (RSD = 1.74)	0.71
	0.027*(MB) - 0.004*(SA) - 0.031*(HUMP) + 54.671 (RSD = 1.50)	0.79
	0.026*(MB) - 0.001(SA) - 0.028*(HUMP) - 0.036*(OSS) + 59.250 (RSD = 1.40)	0.82
	0.026*(MB) + 0.001(SA) - 0.019*(HUMP) - 0.035*(OSS) + 0.158*(RB) + 56.639 (RSD = 1.32)	0.84
RMP131 GRL	0.005*(MB) + 51.985 (RSD = 1.70)	0.10
	0.007*(MB) - 0.005*(SA) + 54.101 (RSD = 1.60)	0.21
	0.008*(MB) - 0.005*(SA) - 0.039*(HUMP) + 56.418 (RSD = 1.17)	0.58
	0.008*(MB) - 0.001(SA) - 0.035*(HUMP) - 0.045*(OSS) + 62.059 (RSD = 0.94)	0.73
	0.007*(MB) + 0.001(SA) - 0.024*(HUMP) - 0.042*(OSS) + 0.192*(RB) + 58.887 (RSD = 0.76)	0.83
RMP131 RST	0.005*(MB) + 60.639 (RSD = 1.71)	0.09
	0.007*(MB) - 0.006*(SA) + 62.810 (RSD = 1.60)	0.21
	0.008*(MB) - 0.005*(SA) - 0.038*(HUMP) + 65.104 (RSD = 1.18)	0.57
	0.007*(MB) - 0.001(SA) - 0.034*(HUMP) - 0.047*(OSS) + 70.979 (RSD = 0.93)	0.73
	0.007*(MB) + 0.001(SA) - 0.024*(HUMP) - 0.044*(OSS) + 0.196*(RB) + 67.751 (RSD = 0.74)	0.83
TFL052 SF	0.021*(MB) + 62.837 (RSD = 1.67)	0.66
	0.022*(MB) - 0.004*(SA) + 64.417 (RSD = 1.61)	0.68
	0.023*(MB) - 0.004*(SA) - 0.024*(HUMP) + 65.890 (RSD = 1.46)	0.74
	0.022*(MB) - 0.001(SA) - 0.021*(HUMP) - 0.035*(OSS) + 70.302 (RSD = 1.36)	0.78
	0.022*(MB) - 0.001(SA) - 0.013*(HUMP) - 0.033*(OSS) + 0.157*(RB) + 67.711 (RSD = 1.28)	0.80
MSA index 5 days	0.011*(MB) + 56.151 (RSD = 1.50)	0.40
	0.013*(MB) - 0.005*(SA) + 58.038 (RSD = 1.41)	0.47
	0.014*(MB) - 0.005*(SA) - 0.030*(HUMP) + 59.840 (RSD = 1.12)	0.66
	0.013*(MB) - 0.001(SA) - 0.026*(HUMP) - 0.041*(OSS) + 65.060 (RSD = 0.93)	0.77
	0.012*(MB) + 0.001(SA) - 0.017*(HUMP) - 0.039*(OSS) + 0.172*(RB) + 62.225 (RSD = 0.78)	0.84

RSD = residual standard deviation.

[1] Predicted MQ4 for five "cut × cooking method" combinations. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 10 days and cooked according to the most common cooking method for each cut; CUB045 GRL = MQ4 score of CUB045 (*Longissimus thoracis*) assuming the grilling cooking method; STA045 GRL = MQ4 score of STA045 (*Longissimus thoracis et Lumborum*, anterior striploin piece) assuming the grilling cooking method; RMP131 GRL = MQ4 score of RMP131 (*Gluteus medius*) assuming the grilling cooking method; RMP131 RST = MQ4 score of RMP131 assuming the roasting cooking method; TFL052 SF = MQ4 score of TFL052 (*Obliquus internus abdominis*) assuming stir-frying cooking method.

[2] MSA index 5 = carcass predicted MSA score calculated as the weighed sum of the predicted MQ4 scores of all MSA cuts. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 5 days and cooked according to the most common cooking method for each cut.

[a] MB = MSA marbling score; SA = slaughter age (days); HUMP = hump height (mm); OSS = ossification score; RB = rib fat depth (mm).

[*] The asterisk indicates that the predictor is significant ($P < 0.05$).

Table 6
Stepwise regression analysis to predict Meat Standards Australia (MSA) predicted meat quality scores (MQ4)[1] of different muscles per cooking method on the basis of MSA traits recorded at the 10th carcass grading site.

MQ4	Equation[a]	R[2]
CUB045 GRL	0.021*(MB) + 57.767 (RSD = 1.38)	0.70
	0.023*(MB) - 0.004*(SA) + 59.242 (RSD = 1.31)	0.73
	0.024*(MB) - 0.004*(SA) - 0.022*(HUMP) + 60.573 (RSD = 1.15)	0.79
	0.023*(MB) - 0.0004(SA) - 0.019*(HUMP) - 0.037*(OSS) + 65.273 (RSD = 1.01)	0.84
	0.022*(MB) - 0.001(SA) - 0.011*(HUMP) - 0.035*(OSS) + 0.152*(RB) + 62.743 (RSD = 0.91)	0.87
STA045 GRL	0.026*(MB) + 50.167 (RSD = 1.73)	0.69
	0.028*(MB) - 0.003*(SA) + 51.477 (RSD = 1.69)	0.70
	0.029*(MB) - 0.003*(SA) - 0.031*(HUMP) + 53.314 (RSD = 1.45)	0.78
	0.028*(MB) + 0.0001(SA) - 0.027*(HUMP) - 0.037*(OSS) + 57.998 (RSD = 1.34)	0.82
	0.027*(MB) + 0.002(SA) - 0.019*(HUMP) - 0.035*(OSS) + 0.151*(RB) + 55.479 (RSD = 1.27)	0.84
RMP131 GRL	0.004*(MB) + 52.113 (RSD = 1.70)	0.06
	0.007*(MB) - 0.005*(SA) + 54.129 (RSD = 1.60)	0.17
	0.008*(MB) - 0.005*(SA) - 0.037*(HUMP) + 56.366 (RSD = 1.20)	0.54
	0.007*(MB) - 0.001(SA) - 0.033*(HUMP) - 0.047*(OSS) + 62.404 (RSD = 0.96)	0.71
	0.006*(MB) + 0.001*(SA) – 0.022*(HUMP) - 0.045*(OSS) + 0.212*(RB) + 58.877 (RSD = 0.73)	0.83
RMP131 RST	0.005*(MB) + 60.534 (RSD = 1.71)	0.07
	0.007*(MB) - 0.005*(SA) + 62.560 (RSD = 1.61)	0.18
	0.008*(MB) - 0.005*(SA) - 0.038*(HUMP) + 64.826 (RSD = 1.21)	0.54
	0.007*(MB) - 0.001(SA) - 0.034*(HUMP) - 0.049*(OSS) + 71.117 (RSD = 0.94)	0.72
	0.006*(MB) + 0.001*(SA) - 0.023*(HUMP) - 0.047*(OSS) + 0.193*(RB) + 67.908 (RSD = 0.76)	0.82
TFL052 SF	0.022*(MB) + 61.971 (RSD = 1.56)	0.67
	0.024*(MB) - 0.004*(SA) + 63.500 (RSD = 1.50)	0.69
	0.025*(MB) - 0.004*(SA) - 0.023*(HUMP) + 64.845 (RSD = 1.37)	0.75
	0.024*(MB) - 0.0004(SA) - 0.019*(HUMP) - 0.039*(OSS) + 69.772 (RSD = 1.23)	0.80
	0.024*(MB) - 0.001(SA) - 0.012*(HUMP) - 0.037*(OSS) + 0.131*(RB) + 67.592 (RSD = 1.17)	0.82
MSA index 5 days	0.011*(MB) + 55.771 (RSD = 1.48)	0.36
	0.013*(MB) - 0.005*(SA) + 57.532 (RSD = 1.39)	0.44
	0.014*(MB) - 0.004*(SA) - 0.030*(HUMP) + 59.308 (RSD = 1.11)	0.64
	0.013*(MB) - 0.001(SA) - 0.026*(HUMP) - 0.043*(OSS) + 64.817 (RSD = 0.89)	0.77
	0.013*(MB) + 0.001(SA) - 0.017*(HUMP) - 0.041*(OSS) + 0.167*(RB) + 62.034 (RSD = 0.75)	0.84

RSD = residual standard deviation.

[1] Predicted MQ4 for five "cut × cooking method" combinations. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 10 days and cooked according to the most common cooking method for each cut; CUB045 GRL = MQ4 score of CUB045 (*Longissimus thoracis*) assuming the grilling cooking method; STA045 GRL = MQ4 score of STA045 (*Longissimus thoracis et Lumborum*, anterior striploin piece) assuming the grilling cooking method; RMP131 GRL = MQ4 score of RMP131 (*Gluteus medius*) assuming the grilling cooking method; RMP131 RST = MQ4 score of RMP131 assuming the roasting cooking method; TFL052 SF = MQ4 score of TFL052 (*Obliquus internus abdominis*) assuming a stir-frying cooking method.

[2] MSA index 5 days = carcass predicted MSA score calculated as the weighed sum of the predicted MQ4 scores of all MSA cuts. The model assumes that all the animals were Achilles hung and all cuts were assumed to be aged for 5 days and cooked according to the most common cooking method for each cut.

[a] MB = MSA marbling score; SA = slaughter age (d); HUMP = hump height (mm); OSS = ossification score; RB = rib fat depth (mm).

[*] The asterisk indicates that the predictor is significant ($P < 0.05$).

marbling trait recorded at the 5th versus 10th carcass grading site respectively. Except for the RMP131 (GRL or RST), the MSA marbling score explained most of the variability of each trait. The MSA marbling score explained 70%, 69%, and 66–67% of the MQ4 variability respectively for CUB045 GRL, STA045 GRL, and TFL052 SF when measured at the 10th or 5th carcass grading sites. Meanwhile, only around 10% of the variability of RMP131 GRL or RST was explained by marbling score for both 10th and 5th carcass grading sites. The other variables added through the stepwise contributed to explain most of the remaining variability reaching a high R^2 (on average 80%). In fact, adding the slaughter age, hump height, ossification, and rib fat depth to the regression model increased the R^2 with a range from 0.80 to 0.87 for TFL052 SF and CUB045 GRL, respectively.

Results of the present study are consistent with those of Liu et al. (2021) who reported that 51% of the variability of the MQ4 score was explained by MSA marbling score only. Thus, some European countries such as France are looking to implement a carcass grading system that considers traits such as marbling. However, the international or national comparison with other studies should consider the different cohort of animals, which differ in terms of maturity, genetic type, feeding strategies, and the possible use of hormonal growth promotants. In the study of Legrand et al. (2013), the high agreement and consistency across the consumers means it should be possible to manage a commercial MSA-like grading system in France. As an example, marbling score is now used to produce beef of a premium French beef brand of the Beauvallet Company, "Or Rouge" exclusively for the Limousine breed.

Moreover, results reported in Tables 5 and 6 suggest the possibility to use the carcass traits (5th and 10th ribs) to accurately predict the beef eating quality traits. Results at the 10th carcass grading site were similar given the high correlation of marbling scores at the 5th and 10th sites, suggesting that the prediction of beef eating quality traits using carcass traits may be applicable at both carcass grading sites. We observed a different contribution of marbling in explaining the variability of the beef eating quality for each "cut × cooking method" combination suggesting that marbling can affect differently meat-eating quality according to which muscle is studied. However, further studies are needed to understand the relationship between marbling and palatability for different cuts.

4. Conclusions

The implementation of the MSA grading scheme is under investigation in some European countries and this is the first study that provides results for the Italian beef industry. This study showed that for French cattle intensively finished in Italy, there could be some differences related to the measure of MSA marbling score according to the carcass grading site (5th or 10th). This is likely to be related to the fat deposition process in interaction with the age of the slaughtered animals. Therefore, a simple adjustment for the MSA model could allow better prediction in Europe, avoiding over- or under-estimation of animals' potential. The market should be more consumer focused. Italy mainly produces lean meat which is not associated with high palatability due to low intramuscular fat content. Moreover, the possibility to implement the MSA grading scheme could be a great opportunity for the Italian beef industry to be innovative. The combined use of MSA grading scheme with objective, automatic, image-based methods could be a solution to increase European beef eating quality. However, since the MSA grading scheme was initially developed in Australia with other animals' categories, different management practices (e.g., diets, growth path) and production systems, it is important to adapt the MSA grading scheme to European conditions.

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CRediT authorship contribution statement

Matteo Santinello: Writing – original draft. **Nicola Rampado:** Data curation. **Mauro Penasa:** Writing – review & editing. **Jean-François Hocquette:** Writing – review & editing. **David Pethick:** Writing – review & editing. **Massimo De Marchi:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2024.109501.

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