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### Relationships between European carcass evaluation and Meat Standards Australia grading scheme applied to young beef cattle

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#### ABSTRACT

The European carcass grading scheme (EUROP) places large emphasis on meat yield and therefore on quantitative traits such as carcass conformation and superficial fat coverage. However, it falls short in considering sensory properties and consumer satisfaction. In contrast, the Meat Standards Australia (MSA) grading scheme considers, among others, animals' ossification, marbling, and ultimate pH as primary indicators of beef eating quality. This study aims to characterize MSA carcass grading scheme applied to the Italian beef production system, considering its significant role in European beef market. The study involved 3204 Charolais, Limousin, and crossbred young bulls and heifers slaughtered in a commercial Italian abattoir. Data collection spanned a broad range of variables, including animal characteristics, MSA traits, and EUROP carcass grading traits. Regardless of the sex of the animal, no significant relationship was observed between MSA traits and EUROP carcass grading scores. Factors such as sex, age, and arrival season at the fattening unit significantly affected most of MSA traits. Females had significantly higher marbling score, and lower ossification score and hump height than males. Animals imported in autumn and winter had significantly lower marbling score, but similar ossification score compared to those imported in spring and summer. Older females had the highest marbling scores. While further research is needed to assess whether the MSA grading scheme can be adapted to all different European rearing systems, results of this study are a prelude to the potential benefits that the MSA grading scheme can bring to the European beef industry.

#### 1. Introduction

The European beef industry grapples with challenges arising from the competition posed by alternative protein sources (Bonny, Gardner, Pethick, & Hocquette, 2015), and growing concerns about environmental impact, animal welfare, food safety, and eating quality (Liu, Ellies-Oury, Stoyanchev and Hocquette, 2022b). Historically, the European beef industry has not given priority to improve meat sensory properties, and producers lack feedback from consumers regarding critical sensory features, such as meat palatability (Bonny et al., 2018a; Hocquette et al., 2018). Indeed, the European carcass grading scheme (EUROP) has been an important tool for pricing carcasses, addressing aspects related only to carcass yield. Consequently, this scheme considers traits which are poorly or not related to the sensory properties of

beef (Monteils et al., 2017; Liu et al., 2020), potentially inducing consumer dissatisfaction. An ideal pricing system should incorporate both carcass yield and eating quality when estimating the value of the carcass (Cross & Savell, 1994; McGilchrist, Polkinghorne, Smith, & Thompson, 2022). Beef carcasses are categorized through EUROP carcass grading scheme based on their conformation and superficial fatness scores (EU 2013/1308, n.d.). European carcass conformation score ranges from E (Excellent) to P (Poor), and fatness score from 1 (very lean) to 5 (very fat). Specifically, these scores consider factors such as muscle development, overall shapes, and superficial fat deposition on the whole carcass. Consequently, genetic breeding programs of beef cattle have traditionally emphasized muscle conformation, growth rate, carcass weight and yield, and low presence of superficial and intramuscular fat, contributing to product standardization (Polkinghorne, Philpott, Gee, Doljanin

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and Innes, 2008a; Hocquette et al., 2018; Liu et al., 2020). However, intramuscular fat content plays a key role in various meat quality traits (Hocquette et al., 2010), rather than superficial fat. This underscores the need for a comprehensive reevaluation of EUROP carcass grading schemes to align with consumer preferences and promoting beef eating quality (Bonny et al., 2018a). Indeed, European consumers are willing to pay a premium for better beef quality but only if the system manages to deliver its promises (Verbeke et al., 2010).

Meat quality is a multifaceted attribute influenced by various factors from the farm to the plate including the breed, sex, and age of the animal, as well as management and feeding strategies, pre-slaughter stress, electrical stimulation, ultimate pH, suspension of the carcass in the chiller, aging time, and cooking method, in addition to biochemical traits such as collagen and fat contents and characteristics which are themselves regulated by most of these factors (Thompson, 2004; Watson, Gee, Polkinghorne and Porter, 2008a; Watson, Polkinghorne, & Thompson, 2008b). The Meat Standards Australia (MSA) system stands out for its comprehensive assessment of beef palatability through preand post-slaughter characteristics. In Australia, MSA evaluation is typically performed 24 h after slaughter at the 10th ribbing site on Longissimus thoracis et lumborum (Polkinghorne & Thompson, 2010). The MSA grading scheme incorporates information on animal and carcass attributes, such as percentage of Bos indicus, hormonal growth implant status, sex, carcass weight, ossification score, marbling score, ultimate pH, carcass suspension methods, days of aging, and cooking methods for different meat cuts. The carcass traits are recorded by trained chiller assessors and used to generate scores for each muscle based on specific aging times and cooking methods, for the prediction of the average consumer sensory experience (Bonny et al., 2018b; Bonny et al., 2018a). A multiple regression approach (MSA model) is used to produce the scores as an outcome from 0 to 100, and categorize each cut using 4 levels, from low to high eating quality (3 stars, 4 stars, 5 stars, respectively), excluding animals with score lower than 46 (fail; Polkinghorne, Thompson, Watson, Gee and Porter, 2008b; Watson et al., 2008a; Meat & Livestock Australia Limited, 2018). The MSA grading scheme not only provides a nuanced understanding of beef quality but also enables a practical and effective means of communicating this information to consumers through a clear, simple, and easy to understand grading system. For instance, the MSA system has developed a simple index (MSA Index) which could also be utilized for benchmarking and to track beef eating quality trends of the carcass at regional, state or national levels (McGilchrist, Polkinghorne, Ball, & Thompson, 2019).

Several studies have assessed the potential usefulness of the MSA grading scheme in Europe (Hocquette, Legrand, Jurie, Pethick, & Micol, 2011; Legrand, Hocquette, Polkinghorne, & Pethick, 2013; Bonny et al., 2016b; Liu et al., 2020; Santinello et al., 2024a), but never applying it on a large scale to Italian beef production system, which ranks as the fourthlargest beef producer in Europe (De Roest, 2015). The Italian beef production system mainly comprises young bulls and heifers of French breeds, and other minor beef breeds and crossbred animals, imported at 10-14 months of age and 300-400 kg of BW (Gallo, De Marchi, & Bittante, 2014; Santinello, Diana, De Marchi, & Penasa, 2020). In contrast, a significant proportion of French beef production is based on cull cows, which are fattened after the end of their reproductive career (Hocquette et al., 2018). These cows, having fulfilled their reproductive duties in a pasture-based system, contribute to the supply chain by providing calves for exportation to other European countries, such as Italy. The particularities of the French production system impact the weight of calves exported seasonally. In general, animals imported in autumn are younger and lighter than animals imported in spring (Dell'Orto & Baldi, 2014). Indeed, animals that are not sold in autumn are kept in the barn with their mothers during winter to be sold in the next spring. During this period, they are typically fed concentrates due to the unavailability of pasture. Once imported, animals are housed in large pens with complete or straw bedding floors and fed total mixed ration once or twice a day. The diet is characterized by a high proportion of concentrates (around 60%) to achieve an average slaughter weight of 750 kg in approximately 6–7 months of fattening (Santinello et al., 2022). Females mature earlier than males and consequently are slaughtered at younger age and lighter BW to prevent excessive fat deposition. Given the disparities in types of animals, production systems, diets, and supply chain structures between Australia and Italy, it is imperative to determine if the MSA carcass grading scheme can be effectively adapted to the Italian beef production system.

A comprehensive analysis of the MSA traits specific to French cattle breeds on a large scale, fattened within the Italian beef production system, is required due to its significant influence on European beef production. Therefore, the aims of this study were to i) characterize performance and MSA traits recorded on 3204 young bulls and heifers in an Italian slaughterhouse, and ii) investigate sources of variation of MSA traits for the most represented breed, i.e., the Charolais (CHL).

#### 2. Materials and methods

Carcass information was retrieved from a commercial slaughterhouse. Therefore, this study did not require an approval from the ethical committee for the care and use of experimental animals.

#### 2.1. Data collection and editing

Data was collected within the framework of the Sustain4Food project, funded by Veneto Region (Venezia, Italy) at the commercial slaughterhouse of the Associazione Zootecnica Veneta (AZoVe, Cittadella, Italy) during the years 2021 and 2022. One certified chiller assessor was trained and accredited by AUS-MEAT to collect data related to the MSA traits and conducted regular recalibrating checks along the study period. The second carcass grader was employed by the slaughterhouse and was trained and certified according to EUROP carcass grading specifications (EU 2013/1308, n.d.). In particular, the MSA grading scheme followed Australian Beef Carcass Chiller Assessment System (ABCAS) and AUS-MEAT Reference Standards (Meat Standards Australia - AUS-MEAT, 2018), and involved the collection of the following MSA traits measured 24 h post-slaughter with carcass chilled at 6-7 °C: 1) ossification score, visually assessed through the degree of calcification in the sacral, lumbar, and thoracic vertebrae (Meat Standards Australia - AUS-MEAT, 2018); 2) carcass hump height (cm), measured from the dorsal point of the hump to the dorsal edge of the Ligamentum nuchae; 3) MSA marbling score (MSA MB), which assesses the presence of marbling through a visual evaluation of the Longissimus thoracis et lumborum muscle at the 5th ribbing site. The MSA MB describes the amount and distribution of intramuscular fat inclusion in the muscle through a scale from 100 to 1190 with 10-point increments; 4) ultimate pH and temperature (Ph meter - RTD Thermometer, Delta OHM, HD2105.1, Italy); 5) hot carcass weight (kg), which excludes kidney and channel fats, diaphragm, tail, as well as trimming excessive fat over topside and brisket cuts.

The EUROP assessor recorded animal ID, date of birth, breed, sex, arrival date in Italy, fattening unit, slaughter date, EUROP carcass conformation and fatness scores. From these data, new traits were calculated, including the arrival age in the Italian fattening unit (days), the length of the fattening cycle (days), the slaughter age (days), the arrival date, and slaughter season. Animals were considered eligible for this study if they had both EUROP and MSA carcass grading information. Since the veterinarians at the slaughterhouse checked the status of internal organs after slaughter process, this information was used to exclude animals from the MSA assessment that were diagnosed injured or had lesions to any internal organs. Specifically, 8 carcasses were discarded due to damage of the Longissimus thoracis et lumborum and other muscles. The remaining 60 carcasses were excluded due to a high incidence of muscle abscesses or signs of respiratory diseases in their lungs, and thus were not evaluated by MSA chiller assessor. Only CHL, Limousin (LIM), and French crossbred (FCR) cattle were considered as

these were the most represented genotypes in the data. Moreover, herds that provided <5 animals during the study were discarded from the dataset (37 animals). Finally, values out of range of animal performance and MSA traits that deviated >3 standard deviations from the respective mean were set to missing values (Table 1). The removed values were out of range for variables of interest.

#### 2.2. Statistical analysis

The distribution of animal performances and MSA traits were visually inspected and found to be normal. Means of performance traits were obtained according to breed and sex. Additionally, means of MSA MB, ossification score, hump height, and ultimate pH were obtained according to the combinations of sex with EUROP carcass conformation and EUROP carcass fatness scores for all the animals, and differences were assessed through one-way ANOVA using Bonferroni post-hoc correction. Subsequently, only CHL animals were retained (1324 females and 1427 males), ensuring the presence of at least 3 animals per slaughter date (45 CHL were removed). The arrival age was categorized according to its mean  $\pm$  0.5 standard deviations to obtain 3 homogenous classes within sex (young female, young male, medium female, medium male, old female, old male). The lower limits for males and females were 279 and 289 days, and the upper limits 331 and 346 days, respectively.

The following linear mixed model was used through the MIXED

procedure of SAS to investigate sources of variation of MSA traits in CHL cattle (2751 animals):

 $y_{ijklm} = \mu + sex_i + age_j + season_k + (sex \ x \ age)_{ij} + (sex \ x \ season)_{ik} + (age \ x \ season)_{ik} + fattening \ unit_l + slaughter_date_m + e_{ijklm}$ 

where  $y_{iiklm}$  is the dependent variable (MSA MB, ossification score, hump height, or ultimate pH);  $\mu$  is the overall intercept of the model;  $sex_i$ is the fixed effect of the *i*th sex of the animal (i = male, female);  $age_i$  is the fixed effect of the *i*th class of arrival age (i = low, medium, high); season<sub>k</sub> is the fixed effect of the kth arrival season (k = autumn, winter, spring, summer); (sex x age); is the fixed interaction effect between sex and class of arrival age; (sex x season)ik is the fixed interaction effect between sex and season of arrival; (age x season)ik is the fixed interaction effect between class of arrival age and arrival season; fattening unit l is the random effect of the lth receiving fattening unit  $\sim N(0, \sigma_{\text{fattening unit}}^2)$ , where  $\sigma_{\text{fattening unit}}^2$  is the fattening unit variance; slaughter\_date<sub>m</sub> is the random effect of the mth date of slaughter  $\sim N(0, \sigma_{\text{slaughter date}}^2)$ , where  $\sigma_{\text{slaugh}}^2$ ter date is the slaughter date variance; and  $e_{ijklm}$  is the random residual  $\sim$  N  $(0, \sigma_e^2)$ , where  $\sigma_e^2$  is the residual variance. Results of the model are presented as least squares means and standard errors, and a multiple comparison of least squares means were performed using the Bonferroni post-hoc test. Significance was set to P < 0.05.

Table 1
Performances and Meat Standards Australia traits<sup>1</sup> recorded on male and female Charolais, Limousin, and French Crossbred cattle.

	Males					Females				
Traits	$n^2$	Mean	$SD^3$	Minimum	Maximum	$n^2$	Mean	SD <sup>3</sup>	Minimum	Maximum
Performance traits										
Charolais	1444					1352				
Arrival age (days)	1428	$305^{\rm b}$	51.9	195	471	1342	318 <sup>a</sup>	57.0	155	493
Length of the fattening cycle (days)	1437	196 <sup>a</sup>	12.7	181	249	1336	194 <sup>b</sup>	11.4	175	244
Slaughter age (days)	1426	$502^{b}$	51.7	383	671	1340	513 <sup>a</sup>	58.9	340	695
Hot carcass weight (kg)	1435	440 <sup>a</sup>	30.1	342	533	1342	$318^{b}$	23.6	241	387
Limousin	38					294				
Arrival age (days)	38	$247^{\mathrm{b}}$	45.5	170	381	292	292 <sup>a</sup>	51.9	170	488
Length of the fattening cycle (days)	35	199 <sup>a</sup>	12.5	185	241	293	192 <sup>b</sup>	10.0	153	236
Slaughter age (days)	37	456 <sup>b</sup>	66.1	369	640	292	484 <sup>a</sup>	52.4	356	677
Hot carcass weight (kg)	36	389 <sup>a</sup>	29.7	349	488	294	301 <sup>b</sup>	21.1	243	371
French Crossbred (n)	27					49				
Arrival age (days)	27	326 <sup>a</sup>	30.8	271	391	48	291 <sup>b</sup>	44.1	207	416
Length of the fattening cycle (days)	27	220 <sup>a</sup>	16.6	188	241	48	193 <sup>b</sup>	8.98	153	213
Slaughter age (days)	27	546 <sup>a</sup>	34.6	483	619	49	491 <sup>b</sup>	58.0	394	693
Hot carcass weight (kg)	27	434 <sup>a</sup>	28.9	387	511	49	314 <sup>b</sup>	23.9	263	363
Meat Standards Australia traits										
Charolais	1444					1352				
MSA MB (score)	1438	368 <sup>b</sup>	68.0	160	560	1333	405 <sup>a</sup>	76.0	200	650
Ossification (score)	1438	177 <sup>a</sup>	18.9	120	230	1335	167 <sup>b</sup>	17.6	110	200
Hump height (cm)	1439	10 <sup>a</sup>	1	6	14	1334	$6^{\mathrm{b}}$	1	3	9
Ultimate pH	1438	5.58	0.08	5.32	5.84	1333	5.57	0.08	5.32	5.82
Limousin	38					294				
MSA MB (score)	38	$322^{b}$	61.4	210	460	294	365 <sup>a</sup>	65.6	200	600
Ossification (score)	38	161	20.6	130	200	293	164	16.2	130	200
Hump height (cm)	37	11 <sup>a</sup>	2	7	13	292	$6^{b}$	1	3	9
Ultimate pH	38	5.55 <sup>a</sup>	0.07	5.38	5.67	293	5.58 <sup>b</sup>	0.08	5.41	5.82
French Crossbred	27					49				
MSA MB (score)	27	$367^{\rm b}$	81.4	210	490	48	417 <sup>a</sup>	79.7	270	610
Ossification (score)	27	189 <sup>a</sup>	11.5	170	230	47	172 <sup>b</sup>	16.3	140	200
Hump height (cm)	27	9 <sup>a</sup>	2	6	12	49	6 <sup>b</sup>	1	4	9
Ultimate pH	27	5.58	0.10	5.42	5.74	49	5.58	0.08	5.45	5.72

a,b For a given trait, means with different superscript letters between sexes within breed differ significantly (*P* < 0.05). Multiple comparisons were performed using the Bonferroni post-hoc test.

<sup>&</sup>lt;sup>1</sup> MSA MB = amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments; Ossification = level of maturity of the animal scored by visual assessment of calcification degree in the *sacral, lumbar,* and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments; Hump height = measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum Nuchae*; Ultimate pH = measured after 24 h of carcass chilling.

 $<sup>^{2}</sup>$  n = number of observations.

 $<sup>^3\,</sup>$  SD = standard deviation.

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#### 3. Results and discussion

#### 3.1. Characterization of performances and MSA grading traits

Animals were transported to Italian fattening units at an average age of 10 months, fattened for approximately 7 months, and subsequently slaughtered before the second year of age (17 months; Table 1), which is a typical practice in North-East of Italy (Diana et al., 2021). The low coefficient of variation for the length of the fattening cycle (~6%) indicated a high level of standardization of the fattening period (Table 1). This standardization is influenced by coupled beef production payments issued under the EU agricultural policy (EU 2021/2115, n.d.), i.e., if an animal is slaughtered after a fattening period exceeding 6 months, the fattener receives a cash premium. Furthermore, there were significant differences in some performance traits based on sex (Table 1). As expected, male animals reached significantly higher hot carcass weight than females (P < 0.05). The study of Santinello et al. (2024b) conducted within the same geographical area using CHL cattle, demonstrated that heifers had lower hot carcass weight than young bulls (323 kg and 444 kg, respectively). Significant variations in performance traits were observed among breeds (results not shown). However, we lacked comprehensive data on the management practices implemented in France and Italy across each fattening unit. This gap could have induced confounding effects, thus prompting us to refrain from delving deeper into breed disparities.

Concerning MSA traits, marbling score was significantly higher in females than in males (+40 marbling points on average; Table 1). This difference could be likely due to the distinct fat deposition mechanism of males and females especially in the muscle (Schumacher, Del Curto-Wyffels, Thomson, & Boles, 2022). Indeed, female cattle have generally higher intramuscular fat deposition compared to males (Venkata Reddy et al., 2015). Liu et al. (2022a) performed a study on Angus x Salers crossbred females and castrated males, reporting no significant differences in MSA marbling score between males and females. The average MSA marbling score in their study was 240 points, which is lower than the average value observed in our work. Indeed, the study of Liu et al. (2022a) involved young animals that were kept at pasture and slaughtered at around 14 months of age. It is likely that this did not allow the deposition of intramuscular fat. The younger age was also confirmed considering the lower ossification score (130 points) and hump height (3.5 cm) reported in the same study. In our work, the observed significant higher hump height and ossification score for males can be explained by sexual dimorphism (hump height: 10 cm and 6 cm for males and females; ossification score: 176 and 168 for males and females). However, these differences are moderate considering that the ossification is evaluated on a scale with 10-point increments. This confirms that despite similar maturity status, females had greater marbling

than males. Indeed, the hormonal status of the animals has an impact on muscle development and growth, and consequently males have lower marbling deposition and higher muscle development than females (i.e., higher carcass weight, hump height, and ossification score) (Park et al., 2002; Tan & Jiang, 2024). Thus, the variations in hormonal profile between males and females easily explain some significant differences between both sexes for performances and MSA traits reported in the present study.

## 3.2. Relationship between EUROP and Australian carcass grading schemes

About 1% of the animals reached the highest score of EUROP carcass conformation (S), with the most frequent category for males being E and for females being U (Table 2). In general, sexual dimorphism explains why males were more prone to grow muscle compared to females, resulting in better EUROP carcass conformation score.

Females had significantly higher MSA MB than males, regardless of EUROP carcass conformation class, with the highest values in class S and U. For males and females, MSA MB values had an inconsistent trend across EUROP carcass conformation classes (Table 2). Thus, the present study failed to find a clear link between marbling score and EUROP carcass conformation score. Our results are in contrast with those reported by Janiszewski, Borzuta, Lisiak, Grzeskowiak, and Powałowski (2017), where 172 Polish Holstein bulls slaughtered at 24 months of age were evaluated using the EUROP carcass grading scheme, comparing their carcass conformation with marbling. The study concluded that marbling was significantly lower in the U carcass conformation class, but the assessment of marbling was based on a different metric compared to our study (ranging from 1 point for marbling not visible to 5 points for high marbling), potentially introducing bias into the comparison. Nogalski, Pogorzelska-Przybyłek, Sobczuk-Szul, and Purwin (2019) examined 198 young, crossbred beef bulls, comprising 67 Holstein-Friesian  $\times$  LIM, 65 Holstein-Friesian  $\times$  Hereford, and 66 Holstein-Friesian × CHL crosses, and observed that carcasses with higher conformation score had lower intramuscular fat content, higher shear force values, and lower juiciness scores. The animals included in the study of Nogalski et al. (2019) were slaughtered at an age which was similar to that of CHL bulls of the present study (17-20 months). However, chemical intramuscular fat content and other meat quality traits were considered instead of marbling. In the literature, some studies suggested that the European carcass conformation score reflects quantitative traits, such as muscularity and yield of the carcass, rather than intramuscular fat content and sensory quality traits (Janiszewski et al., 2017; Nogalski et al., 2019). For instance, Bonny, et al. (2016b) demonstrated that the EUROP carcass conformation score had no relationship with eating quality as it was not related to sensory scores.

**Table 2**Means and standard deviations of Meat Standards Australia (MSA) traits according to sex of the animal and EUROP carcass conformation score<sup>1</sup>.

			•			
Sex	EUROP carcass conformation score	Animals $(n)^2$	MSA MB (score)	Ossification (score)	Hump height (cm)	Ultimate pH
Male	S	21	$363\pm48.9^{bc}$	$177\pm20.3^a$	$12\pm1^{\text{a}}$	$5.65\pm0.07^a$
	E	1356	$368\pm68.5^{\mathrm{b}}$	$178\pm18.6^{\rm a}$	$10\pm1^{\rm b}$	$5.57\pm0.08^{\rm c}$
	U	126	$353 \pm 68.9^{\rm c}$	$166\pm20.9^{\rm b}$	$9\pm1^{ m c}$	$5.60\pm0.08^{\mathrm{b}}$
Female	S	17	$416 \pm 97.9^{a}$	$174\pm16.2^{\rm ab}$	$7\pm1^{\rm d}$	$5.59 \pm 0.06^{\mathrm{cb}}$
	E	424	$388\pm80.2^{\rm ab}$	$166\pm16.8^{\mathrm{b}}$	$6\pm1^{ m d}$	$5.57\pm0.08^{\rm c}$
	U	1234	$402\pm73.4^a$	$166\pm17.6^{\rm b}$	$6\pm1^e$	$5.58\pm0.08^{c}$

 $a_bb,c_cd_e$ Means in the same row for both sexes with different letters within trait differ significantly (P < 0.05). Multiple comparisons were performed using the Bonferroni post-hoc test.

<sup>&</sup>lt;sup>1</sup> EUROP carcass conformation score = European visual assessment of carcass muscling expressed through muscularity classes: S = Superior; E = Excellent; U = Very good; R = Good; O = Fairly good; P = Poor; MSA MB = amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments; Ossification = level of maturity of the animal scored by visual assessment of calcification degree in the *sacral*, *lumbar*, and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments; Hump height = measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum nuchae*; Ultimate pH = measured after 24 h of carcass chilling.

 $<sup>^{2}</sup>$  n = number of observations.

However, Bonny, et al. (2016b) included 455 steers and heifers from France, Poland, Ireland, and Northern Ireland with an average slaughter age of 938 days, which referred to categories of animals older than animals in our study. As expected, ossification score and hump height were significantly higher for males and females of S and E categories (Table 2). As several morphometric traits, EUROP carcass conformation score, ossification score, and hump height increase with age (Bonny et al., 2016c). The pH significantly differed across EUROP carcass conformation classes only for males, with animals of S conformation category having higher values than animals belonging to E and U categories (+0.08 and + 0.05, respectively). However, further speculations are not possible since we did not have more information about animals' diet or stress level at slaughter.

Concerning EUROP carcass fatness score (Table 3), only 0.73% of males were in class 3 compared to 82% of females, confirming that females are more prone to deposit external carcass fat (Venkata Reddy et al., 2015). A significant difference in marbling was evident among females with MSA MB score of 406 and 366 depending on their EUROP carcass fatness score of 3 and 2, respectively. The absence of differences in marbling between EUROP carcass fatness classes 2 and 3 in males (Table 3) may be attributed to the lower number of animals classified in EUROP carcass fatness class 3 (11 animals) respect to 2 (1492 animals). Nogalski et al. (2013) reported that most of 108 crossbred animals (Holstein-Friesian cows crossed with bulls of the LIM, Hereford, or Simmental breeds) and 92 Holstein-Friesians young bulls slaughtered at 21-22 months of age, belonged to EUROP carcass fatness class 2, in agreement with our results. In the same study, the EUROP carcass fatness scores did not align consistently with intramuscular fat content. Indeed, those authors reported that intramuscular fat did not significantly vary among EUROP fatness classes both in crossbred and Holstein Friesian young bulls. Other studies showed that carcasses with high EUROP carcass fatness scores were more prone to develop higher level of marbling and better meat quality characteristics in bulls from crossing LIM bulls with Polish Holstein cows (Daszkiewicz & Wajda, 2000; Daszkiewicz, Wajda, Bak, & Matusevicius, 2003). European carcass fatness score demonstrated a moderate to low level of correlation with marbling score measured by image analysis and chemical intramuscular fat content in 40 Pirenaica yearling bulls (0.49 and 0.29, respectively;

**Table 3**Means and standard deviations of Meat Standards Australia (MSA) traits according to sex of the animal and EUROP carcass fatness score<sup>1</sup>.

Sex	EUROP carcass fatness score	Animals (n) <sup>2</sup>	MSA MB (score)	Ossification (score)	Hump height (cm)	Ultimate pH
Male	3	11	366 ± 86.3 <sup>b</sup>	$175 \pm 13.7^{ab}$	$10 \pm 1^a$	$5.54 \pm 0.09^{b}$
	2	1492	$\begin{matrix} 367 \pm \\ 68.3^{b} \end{matrix}$	$177 \pm \\19.2^a$	$10\ \pm \\ 1^a$	$5.58 \pm 0.08^{a}$
Female	3	1374	$406 \pm 75.9^a$	$167 \pm 17.6^{\mathrm{b}}$	$6\pm 1^b$	$\begin{array}{l} 5.57 \pm \\ 0.08^{ab} \end{array}$
	2	301	$\begin{array}{l} 366 \pm \\ 65.4^{\rm b} \end{array}$	$164 \pm \\16.3^{c}$	$6\pm1^{b}$	$\begin{array}{l} 5.58 \pm \\ 0.08^a \end{array}$

 $<sup>^{</sup>a,b,c}$ Means in the same row for both sexes with different letters within trait differ significantly (P < 0.05). Multiple comparisons were performed using the Bonferroni post-hoc test.

Indurain, Carr, Goni, Insausti, & Beriain, 2009). Conroy, Drennan, Kenny, and McGee (2009) observed a moderate positive correlation between EUROP carcass fatness score and fat depth over Longissimus thoracis et lumborum measured through an ultrasound device during the pre-slaughter in Holstein-Friesian and Aberdeen Angus × Holstein-Friesian cattle. Nogalski et al. (2019) reported that meat quality could be better characterized by EUROP carcass fatness score and that it is affected by the slaughter age in crossbred cattle. Thus, although there is still a level of inaccuracy, the EUROP carcass fatness score can slightly contribute to assess meat quality at least in young French beef heifers reared in Italian fattening units. Ossification score and hump height were not linked to EUROP carcass fatness score and the significant differences reported in the Table 3 reflect the sexual dimorphism. Females in class 2 had significantly lower ossification score compared to females in class 3. Since ossification is assessed on a scale with increments of 10, these variances are not to be deemed pivotal.

As mentioned above, the EUROP carcass grading system considers only a few indicators. The premium French beef brand of the Beauvallet Company "Or Rouge" (Pithiviers, France) considers marbling to determine the commercial values of carcasses only from the LIM breed (Santinello et al., 2024a). Intramuscular fat content estimated through MSA MB score is related to sensory quality of the meat (Hocquette et al., 2010), and thus it could be evaluated for a potential modification of the EUROP carcass grading scheme. A recent study has proposed a pocket near-infrared tool for on-field application at low costs to predict MSA marbling score (Kombolo-Ngah et al., 2023). This could be used for commercial purposes and rapid screening if implemented into a standardized protocol. However, the carcass is a complex heterogeneous entity that includes different muscles (Ellies-Oury et al., 2020), which can have different marbling level and sensory qualities. Given that the MSA grading scheme offers a meat quality score for various combinations of muscles in interaction with cooking methods, this approach could offer valuable insights to improve carcass grading in Europe. Nonetheless, these hypothetical improvements should consider not only beef eating quality but also environmental aspects and nutritional traits, and would need to be easily understand and flexible enough to allow companies to develop their own brand (Farmer & Farrel, 2018).

Considering the relevant role of Italian beef production in Europe, it should be reasonable to conduct consumer-based eating quality assessments in a future study and this is a limitation of our work. Indeed, sensory assessments have been previously conducted successfully in France and Poland on a number of dairy cows and young bulls of different French and Polish breeds (Bonny et al., 2016a; Legrand, Hocquette, Polkinghorne, & Wierzbicki, 2017; Liu et al., 2023). In particular, the study of Legrand et al. (2017) involved 60 animals: 10 cull cows, 11 primiparous cows, and 9 multiparous cows (French animals), and 13 young bulls from dairy breeds, 13 from beef breeds or crossbreeds, and 4 of unknown breed type (Polish animals). Grades attributed to meat by Polish consumers were notably lower than those given by French consumers. Moreover, Legrand et al. (2017) reported significant MQ4 prediction differences based on muscle and consumer origins. Polish MQ4 predictions were largely underestimated, likely due to the model alignment with Australian cattle breeds, which may not accurately reflect young Polish cattle. The study of Liu et al. (2023) reported a lower MQ4 prediction ability of 64.5% compared with the 70% reported by Legrand et al. (2017). The study of Bonny et al. (2016a) involved 482 animals from France, Poland, and Ireland, including beef breeds (Angus, Hereford, Murray Grey, Shorthorn, Belted Galloway, Belgian Blue, CHL, Blonde d'Aquitaine, LIM, Montbeliarde, Romagnola, and Simmental) and dairy breeds (Holstein, Ayrshire, Normande). Those authors reported lower eating quality for bulls than for females, and slightly lower eating quality for beef breeds than for dairy breeds and crossed animals. Thus, it would be interesting to conduct consumerbased eating quality assessments with animals reared in the Italian system in future studies.

 $<sup>^1</sup>$  EUROP carcass fatness score = European visual assessment of superficial fat of the carcass. The EUROP carcass fatness score has 5 categories, from 1 (low presence of superficial fat) to 5 (high presence of superficial fat); MSA MB = amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments; Ossification = level of maturity of the animal scored by visual assessment of calcification degree in the *sacral, lumbar,* and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments; Hump height = measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum nuchae*; Ultimate pH = measured after 24 h of carcass chilling.

 $<sup>^{2}</sup>$  n = number of observations.

#### 3.3. Sources of variation of MSA traits of CHL beef cattle

In Fig. 1, the distributions of MSA traits (MSA MB, ossification score, hump height, and ultimate pH) are presented for CHL cattle (n=2751). The statistical model was carried out for MSA traits only on CHL animals. *F-values* and significance (*P-value*) of fixed effects are presented in Table 4. Sex was the most relevant factor to explain the variability of all MSA traits (P < 0.05), except for ultimate pH, and this could explain why we found little differences in pH values between sexes. Arrival age affected MSA-MB and ossification scores (P < 0.05), and arrival season affected all MSA traits (P < 0.05). The interaction between sex and arrival age was significant to explain the variability of marbling scores (Table 4; P < 0.05). The interaction between arrival season and sex was significant for marbling scores and hump height (P < 0.05), and the interaction between arrival season and arrival age was never significant.

The least squares means of the MSA traits for the sex effect are shown in Table 5. Males had significant lower MSA MB score (males: 368; females: 414) and significant higher ossification score and hump height compared to females. The higher marbling scores agree with the review of Venkata Reddy et al. (2015). Lee, Evans, Nute, Richardson, and Scollan (2009) reported that cows (5.40 points) and heifers (3.70 points) had higher marbling scores compared to bulls (1.50 points) and steers (2.88 points). The differences suggest varying fat deposition patterns exist between sexes as previously discussed. The authors suggested that female cattle possess genes that efficiently control fat deposition, potentially explaining the observed disparities in marbling scores. These results are well supported by data from the US National Beef Quality Audit, which shows that heifers had slightly more marbling (Moore et al., 2012). The higher rate of ossification score and hump height in males can be explained by the higher growth rate of males compared to females (Scheffler, Buskirk, Rust, Cowley, & Doumit, 2003). Choi et al. (2002) reported that heifers exhibit significant lower hot carcass weight and lower measurements for carcass length, suggesting that they generally have low growth rate. The superior performances of bulls are attributed to steroids, primarily testosterone but also estradiol, produced in the testes (Lee, Henricks, Skelley, & Grimes, 1990). Testosterone binds to receptors in muscles, stimulating increased incorporation of amino acids into protein, thereby increasing muscle mass and morphological traits without a concomitant increase in adipose tissue (Dayton & White, 2008). Only ultimate pH was similar between males and females; however, this result is in line with findings presented in Table 1 for CHL animals.

The effects of arrival season on MSA traits are presented in Table 5. Most of the animals arrived in autumn (32%) and summer (28%), and only 16% in spring. Marbling score was significantly higher in animals which arrived in spring and summer compared to autumn and winter. This is likely due to the peculiarities of French production system. Indeed, animals imported in spring and summer are called "Repousse" and are heavier and older compared to animals imported in autumn and winter called "Broutard". Indeed, "Repousse" animals remained in the French barns with their mothers and are usually fed concentrates in winter. This is reflected in their higher MSA MB due to higher body weight after the different feeding strategy and higher age. Similar results were reported by Razminowicz, Kreuzer, and Scheeder (2006). Additionally, higher summer temperature can generate heat stress in the animals and in turn can favor greater muscle marbling and fat deposition in the internal depot rather than in the subcutaneous depot (Mader & Davis, 2004). Ultimate pH was significantly higher in carcasses of animals which were imported in summer and lower for animals imported in other seasons. The observed differences in ultimate pH levels among the carcasses may be attributed to variations in animal feeding behavior influenced by heat stress during summer, potentially affecting muscle glycogen levels due to the reduction of feed intake (Renaudeau et al., 2011). Indeed, fluctuations in feed quality and composition across seasons can impact glycogen levels and indirectly the ultimate pH. However, as information regarding the specific diets provided to each animal

was not available, further research is need to support this hypothesis.

Table 5 also shows the least squares means of the interaction effect between sex and arrival age. Older females (high and medium classes) had the highest MSA MB score while males had the same MSA MB across all the categories of arrival age, aligning with previous studies (Greenwood et al., 2015; Czyżak-Runowska et al., 2017). The general low variability (coefficient of variation = 18%) in animals arrival age and thus in their slaughter age may explain why the present study failed to find significant differences across male classes of arrival age. Additionally, it is worth noting that males tend to direct energy towards muscle growth rather than intramuscular fat deposits. Ossification score decreased as the arrival age group decreased from high to low irrespective of sex, and it was higher for animals in the oldest class of arrival age and decreased through medium and low classes. Hump height was significantly different between males and females, confirming the previously reported differences, whereas pH was the same within and between sexes and classes of arrival age. Similar results were obtained by Lucero-Borja et al. (2014), who observed that pH of different beef cattle categories reared at pasture did not differ significantly.

#### 4. Conclusion

The present study is the first investigation of the MSA grading scheme within the integrated beef production system between Italy and France, and provides valuable insights into the potential application of the MSA grading scheme to young beef cattle in Italy. Our findings support that MSA traits are affected by multiple sources of variation such as sex, age, and arrival season at the fattening unit, thus demonstrating the relevance of these criteria in the Italian context. Moreover, MSA traits are not related to EUROP carcass conformation and fatness scores in young cattle, although it was observed that females classified as EUROP fatness score class 3 had greater marbling score than females in class 2. This suggests the potential utility of incorporating MSA traits such as marbling in addition to EUROP carcass grading system to better enhance the predictive capacity of consumer satisfaction and strengthen the competitiveness of the beef industry. Notably, the development of the MSA model in Australia has added value to the entire beef supplying chain because of better consumer satisfaction (Meat Standards Australia, 2023). Further research is warranted to explore the feasibility of adapting the MSA model to various European rearing systems, with the aim of maximizing benefits for the European beef industry.

#### **Ethic statement**

Ethical review and approval were not required because data were retrieved from a commercial slaughterhouse.

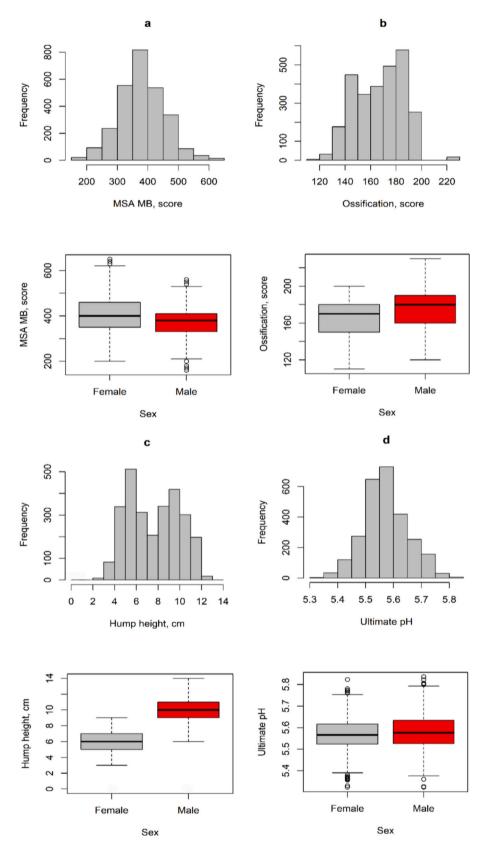
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#### CRediT authorship contribution statement

Matteo Santinello: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Mauro Penasa: Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. Arianna Goi: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Nicola Rampado: Data curation. Jean-François Hocquette: Validation, Supervision, Methodology, Conceptualization. Massimo De Marchi: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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**Fig. 1.** Distribution of Meat Standards Australia (MSA) traits for Charolais cattle (n = 2751) after editing. Traits are: a) MSA MB (amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments); b) Ossification (level of maturity of the animal scored by visual assessment of calcification degree in the *sacral*, *lumbar*, and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments); c) Hump height (measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum nuchae*); and d) Ultimate pH (measured after 24 h of carcass chilling).

**Table 4** *F-values* and significance (*P-value*) of fixed effects of sex, arrival age, arrival season, and their interactions for Meat Standards Australia (MSA) traits of Charolais cattle (*n* = 2751).

Traits <sup>1</sup>	Sex	(S)	Arrival .	Age (AA)	Arrival s	eason (AS)	S x	AA	Sx	AS	AA	x AS
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
MSA MB (score)	144	< 0.001	6.53	0.002	55.4	< 0.001	6.86	0.001	3.35	0.019	0.91	0.49
Ossification (score)	144	< 0.001	64.3	< 0.001	4.46	0.004	1.68	0.19	1.28	0.28	0.67	0.68
Hump height (cm)	3753	< 0.001	1.83	0.16	7.39	< 0.001	0.27	0.76	15.8	< 0.001	2.16	0.05
Ultimate pH	3.65	0.06	0.08	0.93	23.1	< 0.001	0.84	0.43	1.89	0.13	0.87	0.52

<sup>&</sup>lt;sup>1</sup> MSA MB = amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments; Ossification = level of maturity of the animal scored by visual assessment of calcification degree in the *sacral, lumbar*, and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments; Hump height = measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum nuchae*; Ultimate pH = measured after 24 h of carcass chilling.

Table 5
Least squares means and standard errors of Meat Standards Australia (MSA) traits for the effect of sex, arrival season, and the interaction effect between sex and age class of Charolais cattle (n = 2751).

Effects	Levels	Animals	MSA MB	Ossification	Hump height	Ultimate pH
		$(n)^3$	(score)	(score)	(cm)	
Sex	Male	1427	$368 \pm 3.6^{\mathrm{b}}$	$179\pm0.7^{a}$	$10.4 \pm 0.01^{a}$	$5.58 \pm 0.01$
	Female	1324	$414\pm3.6^a$	$168\pm0.7^{\rm b}$	$5.94 \pm 0.01^{b}$	$5.58 \pm 0.01$
Arrival season	Autumn	868	$376\pm4.0^{\rm b}$	$171\pm0.9^{\rm b}$	$8.04\pm0.01^{ab}$	$5.58\pm0.01^{\rm b}$
	Winter	650	$365\pm4.2^{b}$	$173\pm0.9^{ab}$	$7.83\pm0.01^{\rm c}$	$5.57 \pm 0.01^{b}$
	Spring	450	$406\pm4.7^a$	$176\pm1.0^{a}$	$7.91 \pm 0.01^{\mathrm{bc}}$	$5.56\pm0.01^{b}$
	Summer	783	$417\pm4.0^a$	$174\pm0.9^{ab}$	$8.19\pm0.01^{\mathrm{a}}$	$5.61\pm0.01^a$
Arrival age x Sex	Old male	386	$368\pm4.9^{c}$	$186\pm1.1$	$10.1\pm0.01$	$5.59 \pm 0.01$
	Medium male	499	$368\pm4.6^{c}$	$178\pm1.0$	$10.0\pm0.01$	$5.59 \pm 0.01$
	Young male	542	$367\pm4.7^{\rm c}$	$173\pm1.1$	$10.0\pm0.01$	$5.59 \pm 0.01$
	Old female	367	$424\pm4.8^a$	$173\pm1.1$	$6.02\pm0.01$	$5.57\pm0.01$
	Medium female	517	$420\pm4.4^a$	$169\pm1.0$	$5.95\pm0.01$	$5.58 \pm 0.01$
	Young female	440	$398\pm4.8^{b}$	$163\pm1.1$	$5.86\pm0.01$	$5.57 \pm 0.01$

 $<sup>^{</sup>a,b}$ Means with different letters within trait differ significantly (P < 0.05). Multiple comparisons were performed using the Bonferroni post-hoc test.

#### Declaration of competing interest

None.

#### Data availability

Data will be made available upon reasonable request.

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<sup>&</sup>lt;sup>1</sup> MSA MB = amount, size, fineness, and distribution of intramuscular fat inclusion in the muscle scored on a scale from 100 to 1190 with 10-point increments; Ossification = level of maturity of the animal scored by visual assessment of calcification degree in the *sacral, lumbar,* and *thoracic vertebrae* on a scale from 100 to 590 with 10-point increments; Hump height = measured from the most dorsal point of the hump to the dorsal edge of the *Ligamentum nuchae*; Ultimate pH = measured after 24 h of carcass chilling.

 $<sup>^2</sup>$  High = Arrival age > 331 days (26.0% of the animals); Medium = 279 days < Arrival age < 331 days (37.7% of the animals); Low = Arrival age < 279 days (36.3% of the animals).

 $<sup>^{3}</sup>$  n = number of observations.

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