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The marbling of carcases is determined more by the characteristics of the animals themselves than by farming practices

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ABSTRACT

Improved marbling could be of great benefit to the beef industry, responding more effectively to consumer preferences and increasing the market value of the product. To identify the management elements that could determine the degree of marbling of carcases, 128 cows were collected on six different farms, each using different breeding and feeding practices. Multiple statistical tests were carried out to determine whether marbling was more influenced by animal characteristics than by management practices. As expected, within the same farm, the practices used to finish the cows were the same from one animal to the next. We confirm that there was indeed a general level of marbling per farm (highly marbled animals coming from farms 'used' to producing highly marbled animals), without being able to determine the weight of genetic choices or feeding and management practices in this determinism. However, we were able to establish that very high marbled carcases came from heavier slaughtered animals and were associated with (1) management that maximised finishing times and time spent on grass (during the animal's life) and (2) finishing diets rich in maize (grain or silage) and containing flax. The practices and performances associated with low and medium marbling carcases were difficult to separate using the indicators available in this study but were opposite to those of high marbling carcases. This 'overall level of marbling' on the farm makes it possible to prioritise the practices that favour or do not favour the development of marbling on the carcase, and allows to formulate advice to breeders to increase the marbling of their carcases. However, there are still grey areas to be covered to effectively achieve a maximum success rate, which will require further work and a more detailed characterisation of the practices and genetic orientations of the animals.

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Marbling; bovine; carcase; animal performances; multiple correspondence analysis

HIGHLIGHTS

- Heavier animals are more marbled than lighter ones.
- Highly marbled carcases are associated with diets containing maize and/or linseed.
- Management maximising finishing time and time spent on grass is favourable to marbling.

Introduction

Consumers have specific expectations regarding sensory quality (in particular taste and tenderness), health value (fatty acid composition, vitamins, assimilable iron, etc.) (Benjamin and Spener 2009; Field et al. 2009; Verbeke et al. 2010), but also regarding the extrinsic quality of the products they consume (marketing conditions: price, possible differentiation, etc.; production methods: carbon footprint, animal health and welfare, etc.) (Grunert et al. 2004; Ellies-Oury et al. 2019). Among these expectations, the intrinsic quality of beef, and in particular sensory quality, is of paramount importance for the consumer's perception of beef quality.

In Europe, carcase evaluation is based on conformation and fatness, while marbling is not routinely used by abattoirs. However, in France, marbling is an important indicator of the quality of premium carcases in various specifications, particularly for quality labels (Label Rouge and Appelation d'Origine Contrôlée in particular). Indeed, it is generally accepted that a higher level of marbling is actually good for the eating quality of the product (Grunert et al. 2004; Clinquart

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et al. 2022). In fact, different authors (May et al. 1992; Chambaz et al. 2003; Santos et al. 2021) reported that marbling was more closely correlated with juiciness and flavour intensity than backfat thickness in late finishing cattle. Previous results also reported that marbling grade was more closely correlated with tenderness than backfat thickness (Shackelford et al. 1994; Wulf et al. 1996; Shackelford et al. 2001; Chambaz et al. 2003; Bumsted et al. 2012). In addition, Liu et al. found on a sample of over 700 carcases with a wide range of origins, breeds and types of animals, ages, feeding and rearing conditions based on the MSA and EUROP grids that the correlation and relationship between marbling score and European conformation and fat scores was weak and variable depending on the type of animal. While the EUROP grid is good for estimating yield, it does not reflect marbling at all. That's why improving marbling could be of great interest to the beef industry, helping to address declining consumption and providing consumers with better and more consistent beef eating quality (Liu et al. 2020).

Previous research has shown that sensory quality, but also the development of marbling, is determined by a number of factors, depending on the individual characteristics of the animals (breed or genotype, sex, age or physiological maturity, growth potential), the rearing factors applied, particularly during finishing, and the conditions under which the meat is slaughtered and prepared, all of which have a direct or indirect impact on the final organoleptic quality of the beef product (Oury et al. 2007; Clinquart et al. 2022).

The aim of this research was therefore to identify the management elements that can determine the degree of carcase marbling (and to identify a management method that produces marbled carcases). To investigate the determinism of carcase marbling, **our first hypothesis** is that within the same farm, the practices applied to finishing cows are generally the same from one animal to the next, and that these practices determine the overall level of carcase marbling. Our second hypothesis is that this 'overall level of marbling' on the farm will allow us to prioritise the practices that do or don't promote the development of marbling on the carcase and thus formulate advice for breeders.

Material and method

Data collection and farm surveys

In order to highlight the impact of practices on the marbling of carcases, we have identified a set of specifications associated with Label Rouge Limousin, for which marbling is a determining and discerning parameter of quality. Within a single abattoir over a 1 year period, we selected all farms in this specification that had supplied more than 10 Limousin cows, 10 animals being considered sufficient to provide meaningful data. Following this selection, 6 cow-fattening farms were selected, supplying a total of 128 cows. The specifications in question are specific to Limousin cows, requiring the animals to spend a minimum period at grass of 6 months per year during the growth and production periods. In the Label Rouge specifications, the minimum finishing period for animals is set at 60 days. Finishing is based on fodder supplemented by a maximum of 6 kg of cereals and plant proteins (controlled feed and referenced formulas). Although the animals can be finished inside or on grass, in this experiment, they are all finished at the barn to ensure that the marbling of the carcases develops sufficiently. In the specifications, no particular weight gain is expected, but carcases must reach a minimum weight of 300 kg for cows.

Apart from the breed of the animal, there are no restrictions in the Label Rouge specifications regarding the origin of the animals. However, insofar as the data came from a structure located in Nouvelle-Aquitaine, all the animals considered in this work came from Nouvelle-Aquitaine or neighbouring departments.

For each farm, all cows slaughtered at the abattoir between July 2021 and July 2022 were identified. Information on each animal (its identification number, slaughter date) and its performance (carcase weight, age, conformation, fat cover) was recorded after slaughter.

According to the specifications, carcases entering the Label must be lightly marbled, the level of marbling being assessed by cutting. The marbling of these carcases was assessed 24 h after slaughter, at the 5th rib, using a 9-point grading scale by two official AUS-MEAT graders. This internal scale, inspired by and calibrated with the AUS-MEAT grid (Ferguson 2004; Polkinghorne et al. 2008), ranges from 1, representing no marbling, to 9, representing extreme marbling. To insure the robustness, repeatability and quality of the scoring, the evaluation has been done by official AUS-MEAT graders. In order to limit the biases linked to a possible poor assessment of the marbling of intermediate carcases, we decided to categorise the carcases into three groups: those with insufficient marbling (score 4 or below represented as Low), those with just sufficient marbling (scores 5 and 6

Table 1. Selected quantitative and qualitative variables in this study.

Farm characteristics (common to all the cows of a given farm)	Breeding practices (common to all the cows of a given farm)	Animal individual characteristics (different from one cow to another)
• Useful agricultural area of the farm • Farm's main forage area • Calving interval average calving on the farm • Proportion of lightly marbled animals on farm (score of 4 or below) • Proportion of highly marbled animals on the farm (score of 7 or above) • Average slaughter weight of cows on farm	• Average time between decision to slaughter and slaughtering • Average age at weaning • Average age at 1st calving • Proportion of time spent on grass by cows • Length of finishing period • Dehorning (yes / no) • Housing conditions (cubicle housing / straw-bedded) • Composition of the finishing diet: grain corn (yes / no), maize silage (yes / no), linseed (yes / no), high concentrate feed (higher than 6 kg : yes / no) The 'grass finishing' option was not considered, as the cows studied were all finished on a ration distributed at the trough.	• Slaughter age • Born on farm (yes / no) • Slaughter weight • Marbling score (low / medium/ high)

represented as medium), and those with satisfactory marbling (score 7 or above represented as High).

A wide range of information was also collected on each farm between August and December 2022. The survey concerned the farm characteristics (common to all the cows of a given farm), the breeding practices (common to all the cows of a given farm) and the individual characteristics of each cow slaughtered during the study period (different from one cows to another) (Table 1). Among breeding practices, the fattening practices applied to selected animals were particularly detailed: we were mainly interested in the length of the finishing period, the housing conditions (cubicle/straw bedded) and the complete composition of the diet. The finishing diet was then characterised using different variables in order to distinguish the animals according to the characteristic components of their finishing-diet: the presence of grain maize in the finishing diet (yes/no), that of maize silage (yes/no), that of linseed (yes/no) but also the presence of a high level of concentrate feed in the finishing diet (more than 6 kg: yes/no). Other variables (such as whether the animals were finished on grass) were also recorded, but they were not taken into account in the rest of the analysis because they were invariant between the animals (none of the animals in question had been fattened on grass, for example). The slaughter season is therefore a variable which, although recorded, was not included in the analysis because it was not discriminatory.

Statistical approach

The statistical analyses within this study were carried out using the R-Statistics software, R-Studio, version 4.2.1. The primary objective was to identify any relationships between the marbling score and various other variables. To achieve this, different methods were used to analyse both the quantitative and qualitative data sets.

Firstly, for the quantitative data, box plot analysis was used to examine the variation within each variable across different categories of marbling score Appendices A and B. This type of analysis provides a visual representation of how data values are distributed across different levels of the marbling score, helping to identify any apparent associations.

Following the box plot analysis, principal component analysis (PCA) was performed on all quantitative variables. This statistical approach is a commonly used multivariate method designed to simplify the complexity of high dimensional data while preserving trends and patterns. PCA was performed using the FactoMineR package. In addition, the marbling score was included in the PCA as a dummy variable, allowing us to observe potential categories with strong explanatory power and association with the different quantitative variables.

To simplify the visualisation of the quantitative variables with the different categories, a heat map will be generated to summarise the main interaction between the different subcategories of marbling with the highly explanatory variable. This heat map will basically represent the mean of each quantitative variable scaled between the different marbling score categories. Using a technique known as hierarchical clustering, the heat map will help to further break down the connections and relationships between the main categories. This provides a detailed and straightforward overview of the data, making it easier to interpret and understand.

After understanding the positive and negative relationship between the variable and the different marbling scores, a T-test was performed. The T-test is a statistical hypothesis test used to determine whether there is a significant difference between the means of two groups. In this context, it was used to test whether the observed relationships between the marbling score and the other variables were statistically significant. This provides a stronger basis for understanding and interpreting the associations between the marbling score and the other variables considered in this study.

In the next section, we use a technique known as Multiple Correspondence Analysis (MCA) to explore the relationship between the qualitative variables and the marbling score. The MCA was carried out using the FactoMineR package. This statistical approach is considered to be a powerful tool in data analysis for identifying and representing underlying structures in a data set. It provides a means of graphically representing the complex, multidimensional relationships between different categories. In this context, MCA is used to determine how different categories are distributed across the different marbling values. MCA visualises the data in such a way that categories with similar profiles are plotted close together, while those with dissimilar profiles are plotted further apart.

Following the MCA, it is necessary to test whether the high potential variable represents a significant effect. A chi-square or Fisher test is used on the selected variables. To select the test, a contingency table (also known as a cross tabulation table) is a two-dimensional table that shows the frequency distribution of two categorical variables. In these analyses, the Chi-square test is used when the sample size is large and the contingency table is greater than or equal to 5. The Fisher test is used when the sample size is small, and especially when one or more expected cell counts in the contingency table are less than 5. The main objective of this test is to determine if there is a statistically significant association between these variables and their distribution across the different marbling scores.

For the final statistical section, a random forest approach is used to test which quantitative and qualitative variables are best at predicting the marbling score. This technique is a powerful machine learning method known for its resistance to overfitting, mainly due to its nature of multiple decision trees voting to make a final decision. Each decision tree is built using a different bootstrapped sample (a sample taken with replacement) from the training dataset all individuals are sample to test the different selected variables and then measure how much each decision tree's prediction varies due to these changes. By taking the average of these variations across all trees, it is possible to quantify the percentage of importance of each variable in the model.

Results

Descriptive statistics

The 6 farms studied have between 207 and 368 ha of utilised agricultural area, of which the main forage area is between 71 and 100%.

The cows studied spent between 67 and 100% of their lives on grass. The cows studied were weaned between 6.5 and 9.0 months, had their first calving between 26 and 36 months and were slaughtered at an average weight of 493.5 kg after finishing between 5 and 7.5 months (Table 2 left).

During the finishing period, the cows were fed different diets based on

- maize silage and concentrates (farms 1,2&4), forage and concentrates (farm 3)
- wet maize and linseed (farm 5)
- maize silage, concentrates, maize grain and linseed (farm 6)

It can be seen that farms 3, 4 and 5 have a higher proportion of light marbled cows (between 33 and 38%) and a lower proportion of heavy marbled cows (0 to 5%) than the other farms. On the other hand, farm 6 has a high proportion of well marbled cows (19%) and very few light marbled cows (3%). Farms 1 and 2 are characterised by low proportions of heavy marbled cows (5 to 6%) and medium proportions of slightly marbled cows (9 to 19%), reflecting a large majority of cows with medium marbling development.

The majority of the 128 cows considered in this paper were born on the farm where they were subsequently fattened (126/128; Table 2 right). At slaughter (mean age 7.5 ± 1.0 years), their carcases were homogeneous in terms of weight (mean 488.2 ± 32.4 kg). However, the development of marbling was more heterogeneous, with carcases mostly moderately marbled (45%), but also partly very (35%) or very lightly marbled (20%).

Interaction between quantitative variables and marbling scores

The analysis results of the quantitative variables are highly explanatory for the different subcategories of marbling scores. The results of the PCA analyses are

Table 2. Quantitative and qualitative data of the 6 studied farms and the 128 studied cows.

Quantitative and qualitative data of the 6 studied farms (Farm characteristics)

QUANTITATIVE VARIABLES	farm 1	farm 2	farm 3	farm 4	farm 5	farm 6	*Minimum*	*Maximum*	Mean ± SD
Useful agricultural area of the farm SAU (hectares)	207	340	305	368	260	250	207	368	280±55
Farm's main forage area SFP (hectares)	147	308	305	258	224	200	147	308	227±51
Proportion of time spent on grass by cows (%)	100	67	67	75	67	80	67	100	77.4±10.9
Average age at weaning (months)	7.5	6.5	8.0	7.5	9.0	8.5	6.5	9	8.0±0.8
Average age at 1st calving (months)	33	26	36	33	34	36	26	36	33.6±3.0
Calving interval average calving on the farm IVV (days)	365	375	380	375	374	364	364	380	370±6
Length of finishing period (months)	7.5	5.0	6.0	6.0	6.0	6.5	5	7.5	6.3±0.7
Average time between decision to slaughter and slaughtering (days)	7	1	15	14	7	15	1	15	10.7±4.9
Average slaughter weight of cows on farm (kg)	522.2	468.7	477.0	484.1	511.1	488.4	468.7	522.2	493.5±17.0
Proportion of light marbled animals on farm	9	19	**33**	**38**	**36**	3	3	38	20.0±15.0
Proportion of heavy marbled animals on the farm	6	5	0	0	5	19	0	19	8.2±7.6
Age at slaughter (year)	9.32	7.33	6.29	7.78	7.87	6.52	6.29	9.32	7.5±1
Slaughter weight (kg)	522.2	468.7	477.0	484.1	511.1	488.4	468.7	522.2	493.5±17.04

							Total
Number of individual	19	14	10	23	22	40	*128*

QUALITATIVE VARIABLES		farm 1	farm 2	farm 3	farm 4	farm 5	farm 6
Dehorning	yes	18	0	10	23	22	25
	no	1	14	0	0	0	15
Housing conditions	cubicle housing	19	0	0	0	0	40
	straw-bedded	0	14	10	23	22	0
Composition of the finishing diet							
Grain corn	yes	0	0	0	0	22	40
	no	19	14	10	23	0	0
Maize silage	yes	19	14	0	23	0	40
	no	0	0	10	0	22	0
Linseed	yes	0	0	0	0	22	40
	no	19	14	10	23	0	0
High concentrate feed	yes	19	14	10	23	0	40
	no	0	0	0	0	22	0

Data of the 128 studied cows (Animal individual characteristics)

Quantitative variables	Mean ± SD	*Min*	*Max*
Slaughter age (years)	7.71 ± 3.2	2.47	13.87
Slaughter weight (kg)	488.2 ± 32.4	426.49	615

Qualitative variables

Born on farm	yes	126
	no	2
Marbling score	low	26
	medium	57
	high	45

presented in Figure 1. The first two dimensions accounted for 61% of the total variability.

The first dimension accounted for 44.2%, with factors such as finishing period, part-time access to grass, final weight and a high marbling score having a significant positive representation. Conversely, variables such as Agricultural area of the farm, Calving interval average, Farm's main forage area and light marbling, associated with low and medium marbling scores, showed a negative correlation.

The second dimension, accounting for 17% of the variability, was positively represented by variables such as weaning, calving age and time between decision and slaughter. On the other hand, slaughter age and average slaughter weight showed negative correlations.

To explore each category further and to identify a pattern between variables and marbling score, a heat map combined with hierarchical clustering was generated (Figure 2). The results grouped the quantitative variables into two subgroups. The first subgroup included variables such as age at slaughter, light marbling, Agricultural area of the farm, Farm's main forage area and Calving interval average. These showed a positive relationship with medium and low marbling scores and a negative relationship with high marbling scores, except for slaughter age which showed a negative correlation with both medium and high marbling scores.

The second subgroup highlighted variables such as average carcase weight, finishing period, part-time grass access, final weight, high marbling, time from decision to slaughter, weaning, calving age and slaughter age. These variables were mainly negatively associated with medium and low marbling scores, whereas they were positively associated with high marbling scores. From this observation it was clear that medium and low marbling scores were more similar to each other than high marbling scores.

In order to highlight the significant differences between these three categories, a summary of pairwise t-tests was performed between the three marbling score categories Table 3. The results of these analyses showed significant effects mainly when comparing high scores with medium and low scores, except for variables such as calving age, time between decision and slaughter and slaughter age. Conversely, when comparing medium and low marbling scores, a significant effect was only found for part-time access to grass, final weight, light and heavy marbling and

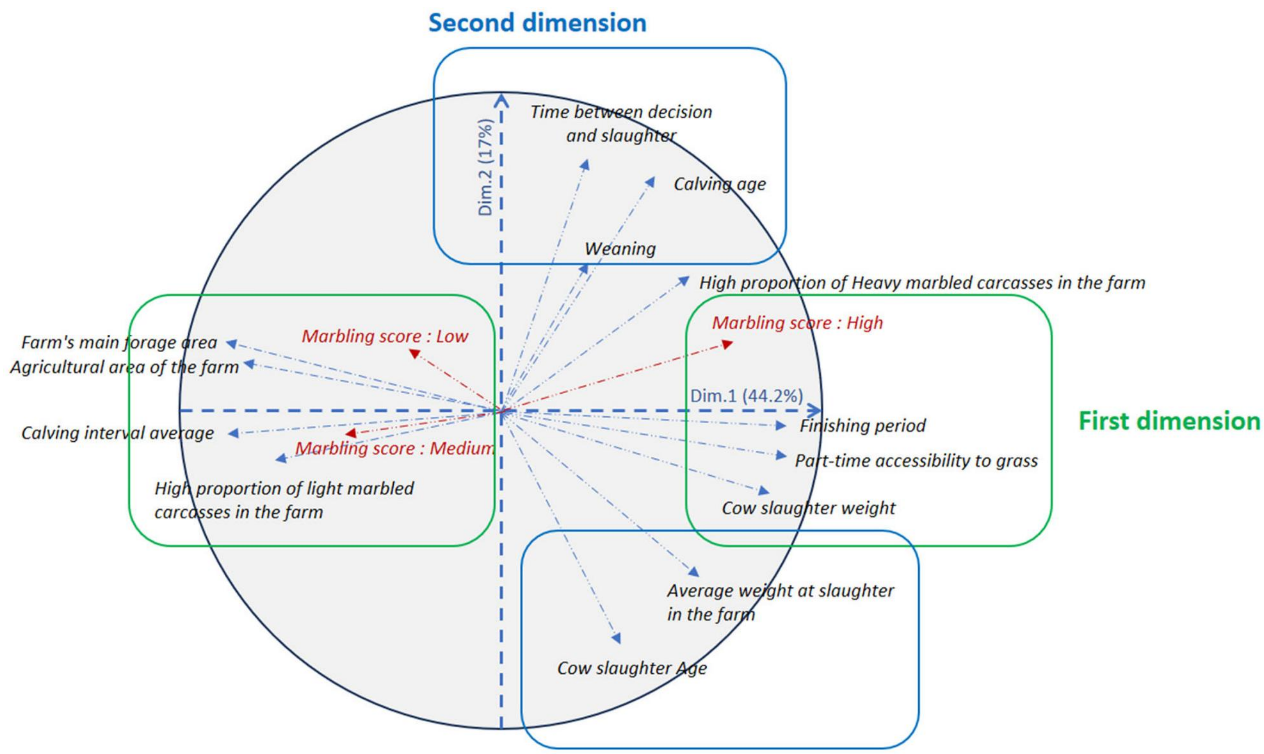

Figure 1. PCA representation of quantitative variables and marbling score dummy variables.
The variables in bold refer to animal characteristics, while those in black refer to farm characteristics.

the Calving interval average variable. The first dimension accounted for 44.2%, with factors such as finishing period, part-time access to grass, final weight and a high marbling score having a significant positive representation. Conversely, variables such as Agricultural area of the farm, Calving interval average, Farm's main forage area and light marbling, which are associated with low and medium marbling scores, showed a negative correlation.

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Interplay between qualitative variables and marbling scores

Multiple Correspondence Analysis (MCA) is a statistical technique used to analyse the categorical data in this dataset, as shown in Figure 3 and Table 4. The first two components, Dimension 1 (30%) and Dimension 2

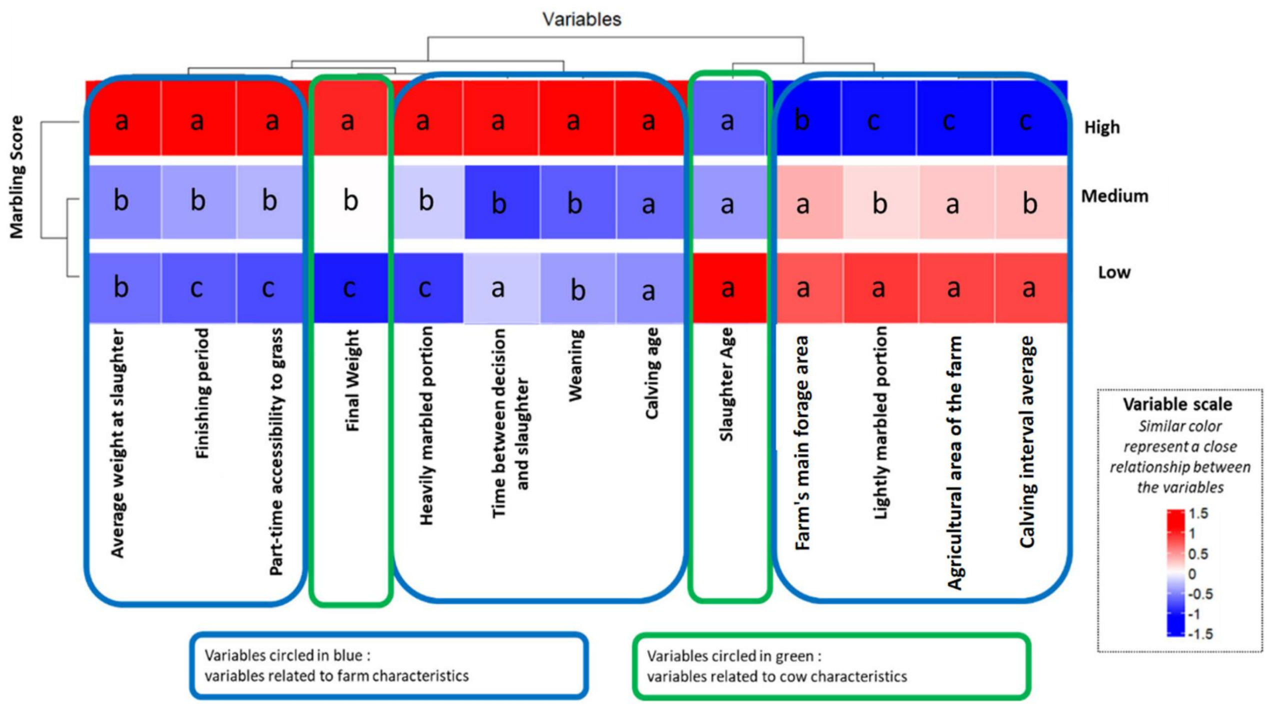

Figure 2. Heatmap representation of quantitative variables across the marbling categories.

Table 3. Pairwise T-test representing the quantitative variables across the different marbling categories.

	Marbling score: high vs low	Sig	Marbling score: high vs medium	Sig	Marbling score: medium vs low	Sig
Slaughter weight	0.018	*	0.01	*	0.85	
Average slaughter weight of cows on farm	<2e-16	***	2.1e-13	***	2.4e-11	***
Slaughter age	0.98		0.98		0.98	
Proportion of light marbled animals on farm : score of 30- or less	<2e-16	***	9.6e-14	***	6.2e-05	***
Proportion of heavy marbled animals on the farm : score of 40- or 40=	4.3e-13	***	5.3e-10	***	4.5e-3	*
Average time between decision to slaughter and slaughtering	0.24		0.03	*	0.48	
Calving interval average IVV	<2e-16	***	<2e-16	***	0.01	***
Average age at 1st calving	0.18		0.072		0.76	
Average age at weaning	6.13e-2	*	3.4e-4	***	0.75	
Farm's main forage area SFP	7.5e-10	***	3.5e-09	***	0.08	
Useful agricultural area of the farm SAU	8.4e-12	***	1.2e-11	***	0.09	
Proportion of time spent on grass by cows	3.7e-12	***	5.1e-10	***	0.01	*
Length of finishing period	1.4e-09	***	1.0e-09	***	0.25	

(*) represents $p < 0.05$, denoting a statistically significant difference.
(***) represents $p < 0.001$, denoting a highly statistically significant difference.
blank cell indicates a non-significant difference ($p > 0.05$).

(29.2%), together account for 59.2% of the total variability.

The correlation circle in the analysis shows clear differences between the high and low marbling groups. Meanwhile, the medium marbling group represents an intermediate category, with some individuals exhibiting characteristics of both high and low marbling.

The graph shows that animals reared in cubicles have higher marbling scores than those reared in straw. In addition, animals that are not dehorned have higher marbling scores than those that are dehorned. Furthermore, animals fed maize, silage and mixed concentrates show a greater accumulation of marbling. Finally, animals born on the farm have a higher marbling score than those born elsewhere.

The contingency table for the variable 'Dehorning' in Table 4 shows that 'Lin', 'Maize' has a frequency greater than 5 and is therefore suitable for the chi-square test. However, the variables 'Born on farm', 'Housing condition', 'Silage' and 'Concentrate' have a frequency lower than 5, making it necessary to use Fisher's exact test to analyse their association with the marbling score.

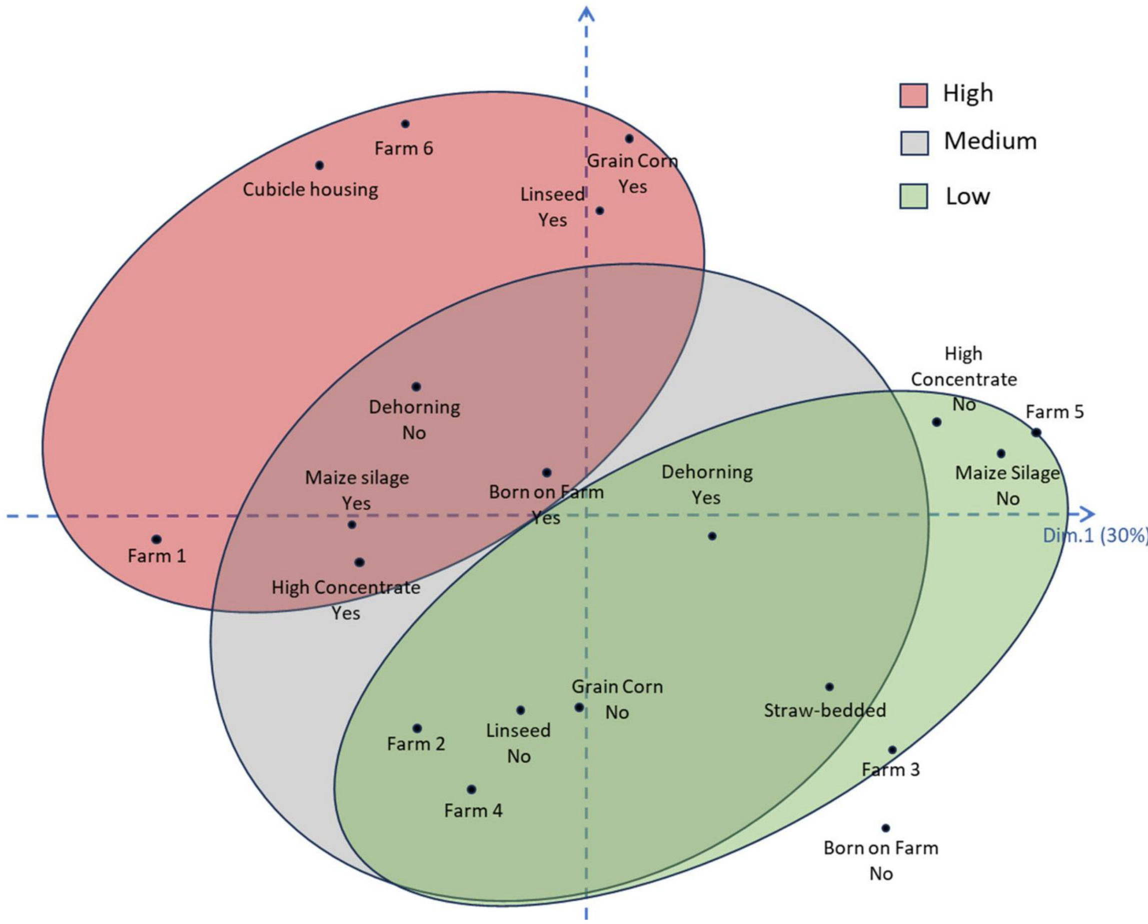

Figure 3. MCA representation of qualitative variables grouped by marbling score.

Table 4. Fisher or Chi2 test representing the qualitative variables across the different marbling categories.

		Marbling rank				
Variable		Low	Medium	High	Test	*p* value
Dehorning	YES	33	23	42	Chi2	0.27
	NO	12	3	15		
High concentrate	YES	43	18	45	Fisher	0.00
	NO	2	8	12		
Maize silage	YES	43	14	39	Fisher	0.00
	NO	2	12	18		
Linseed	YES	31	8	23	Chi2	0.00
	NO	14	18	34		
Housing	Cubicle housing	2	26	41	Fisher	0.00
	Straw-bedded	43	0	16		
Grain corn	YES	31	8	23	Chi2	0.00
	NO	14	18	34		
Born on farm	YES	45	25	56	Fisher	0.4881
	NO	0	1	1		

The cross box represents categories eliminated from the analyses.

Certain subcategories, such as low marbling scores in relation to the variable ‘Born on farm’ and medium marbling scores in relation to the variable ‘Housing’, were excluded from the analysis because their frequency in the contingency table was 0.

The results in Table 4 show a significant effect when analysing marbling scores with the categories ‘Dehorning’, ‘Concentrate’, ‘Silage’, ‘Lin’, ‘Housing’, ‘Maize’ and ‘Born on Farm’. Conversely, no significant effect was observed for the variables ‘Born on farm’ and ‘Dehorning’.

Key variable driving marbling score predictions with random forest

The results of the random forest algorithm show a high potential accuracy for the model with an out-of-bag

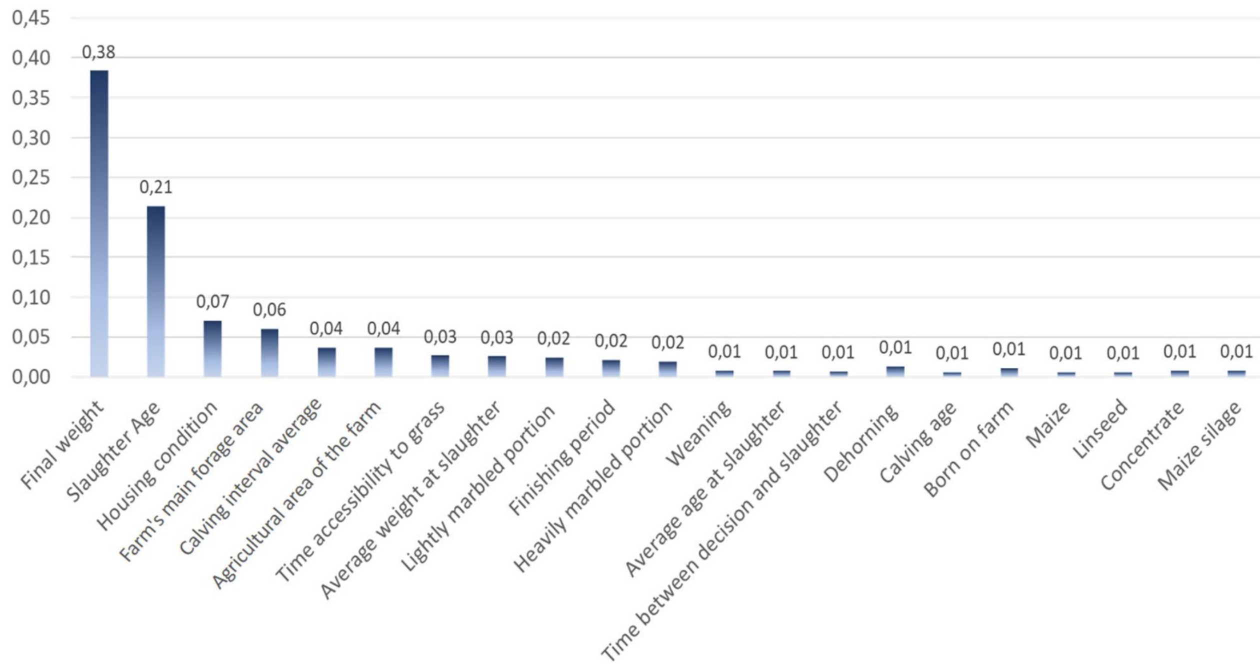

Figure 4. A plot of the mean decrease in precision and the mean decrease in gini to rank the variables according to their importance in explaining and predicting the variability of the marbling score.

(OOB) accuracy of 71.43%. This is a good validation of the predictive power of the model. A ranking of the variables based on their importance, is shown in Figure 4. The results show that final weight and slaughter age are the main explanatory variables for the variability in marbling score. Variables such as housing condition, Calving interval average, Agricultural area of the farm, Farm's main forage area, housing conditions, proportion of time spent on grass, and Finishing period as well as light and heavy marbling, play a secondary role and are considered to be significantly less decisive. On the other hand, factors related to the animal's diet, dehorning practices and whether the animal was born on the farm show a minimal relationship with the marbling score.

Discussion

Statistical approach

Various statistical methods were used to investigate the relationship between husbandry practices and carcase characteristics on marbling characteristics. PCA was used to represent the broad associations between qualitative variables (Kassambara 2017). By transforming the marbling score into a categorical (dummy) variable, a clearer observation is made to determine the relationship between marbling and the other quantitative variables. This approach is considered sufficient as 61% of the total variability between the total quantitative variables is considered a very good score. The result showed that each marbling score had a robust relationship with a group of variables.

To have a better understanding of the relationship between the different subgroups, a scaled heatmap is considered an optimal tool (Wilkinson and Friendly 2009). It provides a visual representation that captures the nuanced interactions between different marbling scores and the quantitative agronomic variables. This allows the identification of the most influential agronomic variables, grouped by their impact on specific marbling grades.

For the qualitative variables, Multiple Correspondence Analysis (MCA) was used and the results showed that a substantial 59% of the variability could be represented. MCA is characterised by its ability to graphically illustrate the sophisticated, multidimensional relationships that exist between different categories (Kassambara 2017). What makes MCA particularly insightful is its ability to represent data spatially. Here, categories with similar profiles or characteristics cluster closely on the plotted graph. In contrast, those with different characteristics are further apart, allowing a clear visual distinction between different categorical relationships.

While pattern recognition and clustering of variables can provide valuable insights, these methods don't always confirm statistically significant relationships. For a more rigorous evaluation, the Chi-square test and Fisher's exact test were chosen for qualitative variables. The Chi-square test examines associations between categorical variables by comparing observed and expected frequencies. For smaller sample sizes or expected frequencies below 5 in any contingency table cell, Fisher's exact test provides a more accurate assessment (Camilli 1995). For quantitative variables, the T-test was used. By integrating these tests, the analysis not only identifies patterns but also validates them, ensuring that conclusions are supported by rigorous statistical evidence.

The Random Forest algorithm was integrated into the analysis because of several key strengths (Ayyadevara 2018). Firstly, it is a very efficient tool for dealing with low repetition scenarios. One of the challenges in this dataset is the presence of categories in qualitative variables that have a low frequency of occurrence. Such scenarios can lead to overfitting in many models, meaning that the model may perform exceptionally well on the training data, but fail to generalise well on new, unseen data. Random forest mitigates this by bootstrapping the data; it creates multiple samples from the original data set by sampling with replacement. This provides a more diverse training process, as each decision tree in the forest sees a slightly different sample, reducing the risk of overfitting. This tool can also rank the data based on their importance in making accurate predictions. For this study, it can effectively identify which farming practices or conditions are most influential in determining marbling scores. Each tree in the forest makes its decision based on a subset of variables, and the algorithm keeps track of how much each variable improves the purity of the decision. These improvements are averaged across all trees, and variables that consistently improve decisions rise to the top in terms of importance.

What determines the marbling of carcases?

In this work we have tried to identify the management elements that can determine the degree of marbling of carcases. In line with our expectations, it was found that within the same farm, the practices used to finish cows were the same from one animal to the next. We were able to confirm that there was indeed a general level of marbling per farm (highly marbled animals coming from farms 'used' to producing highly marbled animals), without being able to determine the weight of genetic choices or feeding and management practices in this determinism. However, we were able to establish that carcases with very high marbling came from heavier slaughtered animals and were associated with (1) management that maximised finishing times and time spent on grass (during the animal's life) and (2) finishing diets rich in maize (grain or silage) and containing flax. It turned out that the practices and performances associated with low and medium marbling carcases were the opposite. In fact, low and medium marbling carcases were difficult to separate using the indicators available in this study. Although our second hypothesis, namely that this 'overall level of marbling' on the farm makes it possible to prioritise the practices that favour or do not favour the development of marbling on the carcase, is validated and we can indeed formulate advice to breeders to increase the marbling of their carcases, there are still grey areas to be covered if we want to effectively achieve a maximum success rate, which will require further work and a more detailed characterisation of the practices and genetic orientations of the animals.

We have been able to demonstrate a relationship between finishing length and carcase marbling, with an increase in finishing length also being associated in our work with an increase in carcase weight at slaughter. Marbling can be defined as 'the intermingling (intramuscular fat) and distribution of fat within the lean meat' (Seggern et al. 2005). It is determined by visual assessment in the *Longissimus thoracis* muscle. To the best of our knowledge, there are no studies in the literature that have established a relationship between finishing length and carcase marbling (whatever assessment grid is used, including the AUS-MEAT grid) on cows, but there are numerous studies that have established a positive relationship between finishing length and carcase and meat adiposity on younger animals, as assessed by carcase EUROP fat cover, carcase fat content or muscle lipid content (Oddy 2003; Dunshea et al. 2005; Scollan et al. 2011). These relationships are particularly linked to a positive relationship between finishing time and growth rate, higher growth resulting commonly in increasing fat thickness and intramuscular (marbling) fat content at the same weight (Oddy 2003). However, it is important to note at this stage that the EUROP fat cover score is not necessarily a good indicator of the degree of the AUST-MEAT marbling score of carcases (Liu et al. 2020). Indeed, according these authors, European classification scores explain only a slight proportion of the variance in marbling score (32%, 46%, 34% and 21% for the entire cattle group, young bulls, females and steers, respectively).

Longer finishing times generally result in higher fat cover (assessed by EUROP grading) (Keane et al. 2006; Vestergaard et al. 2007) and a higher proportion of carcase fat (Dumont et al. 1991; Dumont et al. 1997), although not all authors show these effects to be significant (Malterre et al. 1989; Franco et al. 2009). Similarly, in the literature review by Oury et al. an increase in finishing time is associated with a significant increase in total muscle lipid content (M. Oury et al. 2007), up to 74% in Hereford cattle (Dinius and Cross 1978).

No significant effect of age at slaughter

According to the literature, age at slaughter is likely to have effects on carcase fat development, with these effects differing according to the type of animal considered (Soulat et al. 2021). For example, up to the age of 18 months, later slaughter of young cattle results in a higher carcase fat cover score (Bureš and Bartoň 2012; Marti et al. 2013). Unlike young cattle, carcase fat cover always increases significantly with age at slaughter in heifers (Ahnström et al. 2012; Bureš and Bartoň 2012). Moreover, previous results indicated that age had no significant correlation with carcase fatness traits but is positively correlated with marbling ($r = 0.21$) (Liu et al. 2020). In our case, age appears to be not significantly linked to marbling grade. This can be attributed to the fact that our sample is made up of cows at the end of their career, and therefore rather old, for whom growth and development have come to an end.

Weight, an important determinant in limousine cattle

In our work, an increase in finishing time was associated with an increase in animal weight. Thus, weight is directly and/or indirectly related to the degree of marbling of Limousine cow carcases. In the literature, Ellies-Oury et al. show no significant effect of an increase in live weight on either fat cover or the proportion of adipose tissue in Charolais carcases (Ellies-Oury et al. 2012). On the contrary, Keane et al. found a significant increase in carcase fatness with increasing live weight in beef x dairy crossbred animals (Keane et al. 2006). Do Prado et al. also found significant effects of increased pre-slaughter weight on carcase composition in 1/2 Puruna vs. 1/2 Canchim animals (do Prado et al. 2015), suggesting a variable effect of weight on carcase fat development depending on the breed considered (Soulat et al. 2021).

The role of finishing diets (maize and linseed)

In this work, diet has emerged as a factor likely to influence carcase marbling, with maize and flax based diets being preferred. The favourable effect of maize (and maize silage in particular) on intramuscular fat content has already been widely demonstrated, especially in comparison with hay or grass silage (Listrat et al. 1999; Juniper et al. 2005; Oury et al. 2007; Aviles et al. 2014). On the other hand, the effect of flax on the quantitative development of fat has been less studied (as opposed to the qualitative development, which has been widely studied for this type of ration (Tripathi et al. 2013; Doreau and Ferlay 2015). Nevertheless, it has previously been shown that the addition of linseed to the ration of cull cows led to a 29% increase in the average thickness of back fat, without specifying the effects on intramuscular lipid content or fat cover score (Hernández-Calva et al. 2011). However, this effect has not always been demonstrated, as replacing part of the concentrate diet with extruded linseed had no effect on carcase characteristics in dairy bulls of different breeds (Holstein, Holstein-Friesian, Norwegian, Norwegian × Holstein-Friesian, Holstein × Norwegian, Jersey × Holstein-Friesian) (Dawson et al. 2010; Albertí et al. 2013).

Conclusion

First and foremost, it is important to point out that the conclusions reached in this study relate specifically to Limousin suckler cows, which have alternated between periods at grass and at the trough during their career and have been finished at the trough before slaughter. The majority of the studies on which we can base our results are based on different breeds, younger animals and less extensive farming methods, so it will be necessary to confirm the results obtained here *via* other experiments carried out under similar conditions.

This work confirmed that certain practices and genetic choices were likely to result in an overall level of marbling, with highly marbled animals coming from farms 'used' to producing highly marbled animals. In terms of management, increased finishing time and slaughter weight were likely to favour carcase marbling, as were maize and linseed based rations. While the beneficial effect of flaxseed on the omega-3 fatty acid composition of meat is widely known and promoted in different sectors, the effect of flaxseed on carcase marbling needs to be clarified, as this food is likely to lead to a better response to the expectations of the sector (high marbling), but also of the consumer (tasty, health-promoting meat *via* its omega-3 composition).

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Authors' contribution

MPEO: conceptualisation; funding acquisition; investigation; methodology; project administration; resources; formal analysis; data curation; supervision; validation; visualisation; writing – original draft; writing – review & editing. JA: formal analysis; data curation; software; supervision; validation; visualisation; writing – original draft; writing – review & editing. KI: investigation; methodology; project administration; formal analysis; data curation; supervision; validation; writing – review & editing.

Data and model availability statement

None of the data was deposited in an official repository. Data are confidential but available to reviewers upon request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethics approval

The breeders contacted gave their explicit, informed consent to take part in the survey, and their personal data were protected. In fact, after being informed of the objectives of the study and how the information provided would be used, all the breeders gave their informed consent to the inclusion of their responses before and after their participation in the study.

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