



Machine learning strategy for light lamb carcass classification using meat biomarkers

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ABSTRACT

In Mediterranean areas, lamb meat is considered to be of great commercial value. Moreover, consumers are becoming increasingly interested in understanding the origin of lamb meat and its associated production and breeding systems. Among many applications, algorithms based on artificial intelligence are used to identify the origin of food products, and in this context, algorithms such as the Support Vector Machine (SVM), K-Nearest Neighbours (KNN), and the Artificial Neural Network (ANN) have been proposed to differentiate the origin of the animals according to their feeding diet. The objective of this study was to evaluate the performance of a variable reduction method based on a multiple regression model and three widely-used machine learning algorithms (SVM, KNN and ANN) for the classification of three commercial light lamb carcasses, from three feeding diets, in an indigenous Spanish breed (Mallorquina), using fatty acid and volatile compound biomarkers of meat. Machine learning algorithms were employed to discriminate lamb carcasses using 14 identified significant biomarkers, which were arranged based on an estimation of the relative importance (stepwise forward multiple regression F-score) of the input variables. We achieved high performances for the SVM, KNN and ANN algorithms, with 86%, 98% and 98% prediction accuracy, respectively. Among the 14 biomarkers used, 7 were identified as showing the highest discriminant capacity. The F-scores indicate that C17:1 and C20:5 n-3 fatty acids, and 2,5-dimethylpyrazine and 3-methylbutanal volatile compounds are the four most relevant biomarkers for predicting three lamb feeding diets.

1. Introduction

The lamb meat market is highly demanding in terms of the quality product and the traceability of the system production (Gracia & De-Magistris, 2013). In fact, each market has its preferences when choosing a specific meat product. While, in Northern Europe, consumers prefer meat from heavy lambs, in the Mediterranean countries of Europe, carcasses from light-weight animals are favoured (Campo et al., 2021). In these Mediterranean areas, consumers choose mainly two types of lamb meat: the consumers' first choice is meat from suckling lambs slaughtered at one month of age and raised only on mother's milk, and in second place, light lambs slaughtered around three months old

("Ternasco" category), which are raised mainly on forage or grass and concentrate diets (Ferrer-Pérez & Gil, 2019).

Recently, consumers have become increasingly concerned about intensive meat production systems and the potential environmental harm they cause to the welfare of animals and human health. In this context, while concentrate-based feeding is related to more intensive production systems, other alternatives based on pastoral feeding are associated with traditional production systems which are more respectful with environmental and animal welfare (Montossi et al., 2013). In addition to the green image perceived by consumers of traditional sheep farming methods based on grazing and natural resource utilization (Carrasco et al., 2009), it has been confirmed that

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pasture-based diets can produce healthier lamb meat with higher levels of conjugated linoleic acids and polyunsaturated fatty acids (PUFA) n-3 in fat, and lower levels of saturated fatty acids (SFA) compared to lambs fed on concentrate-based diets (Nuernberg et al., 2008). Generally, ruminants that consume pasture diets have been shown to produce a more desirable fatty acid composition than those fed on grain (Howes et al., 2015). In addition to the effects on meat quality related to fat, flavour is another quality attribute of lamb meat that plays a key role in consumer acceptability (Young et al., 2003). However, Watkins et al. (2013) showed that the effect of feeding regimes on the profile of volatile compounds responsible for aroma is complex, and it is a challenging task to predict the traits that contribute to the flavour of lamb meat. Historically, science has focused on the so-called 'mutton' and 'pastoral' flavours. Thus, the literature clearly associates pastoral flavour with compounds such as 3-methylindole (Young et al., 2003) and other volatile compounds like short-branched-chain fatty acids (BCFA), primarily represented by 4-ethyl-octanoic acid, which are associated with concentrate feeding. These BCFA accumulate in the fat of animals as they age, contributing to the characteristic mutton flavour (Watkins et al., 2013).

Due to the growing consumer demand for clear information about the feed given to animals and the industry's growing need to certify the quality of meat (Biglia et al., 2022), both the meat industry and the administrations are seeking tools to guarantee the product origin and prevent potential consumer fraud. To achieve this, over the last few years, the use of specific meat biomarkers to ensure the traceability of meat production systems has been proposed (Sivadier et al., 2008). It was reported that the feeding regimes of farm animals have effects on the fatty acid profile (Díaz et al., 2005) and volatile compounds in ruminants' meat (Watkins et al., 2013). In fact, the fatty acid profile and volatile compound content of lamb meat have been proposed as potential biomarkers to identify the feeding diet based on the use of milk, grass or concentrate in ruminant meat (Cabiddu et al., 2022; Elgersma, 2015).

Usually, the classification of lamb carcasses in Europe is carried out by trained personnel based on visual observation criteria, such as morphology, degree of fatness, or colour (Russo et al., 2003). However, due to consumer demand for quality meat based on dietary, organoleptic, and technological properties, science is developing new classification methods for carcasses which are more effective than the traditional methods, including the combined use of foodomics with multivariate statistical approaches to classify lamb carcasses according to animal sex (Santos et al., 2007), breed (Ciliberti et al., 2021), geographical origins (Sun et al., 2012) or feeding systems (Wang et al., 2022). In this context, traditional statistical analyses, such as Principal Component Analysis, Discriminant Analysis, or Partial Least Squares Regression, have been common techniques for performing and reducing variables in multivariate data analysis, and they are widely used as a tool in exploratory analysis for various meat classification studies (Guo et al., 2016).

Recently, machine learning has emerged as a powerful tool for conducting food authentication using biochemical food data (Jiménez-Carvelo et al., 2019; Qi et al., 2021). In fact, cutting-edge scientific methods, like the use of machine learning algorithms, such as Support Vector Machine, K-Nearest Neighbours, Artificial Neural Network and other algorithms, exhibit advantages compared to traditional classification methods of food, as has been shown by De Nadai Fernandes et al. (2020) in beef meat, Qi et al. (2021) in pork meat or Frizzarin et al. (2021) in cow milk. In the particular case of lamb, several studies have focused on meat classification using machine learning algorithms. Examples include the research conducted by Sanz et al. (2016) on classifying lamb muscle, Fowler et al. (2021) predicting intramuscular fat content in lamb meat, or Alaiz-Rodríguez and Parnell (2020) assessing lamb meat quality. While traditional statistical analyses for discrimination are limited to a model based solely on a database, machine learning algorithms can mine information from the data itself,

recognize hidden patterns and show more clearly the natural significance of data (Ge et al., 2017; Maione et al., 2019).

The objective of this study was to evaluate the performance of a combined method of variable reduction and three machine learning algorithms (SVM, KNN, and ANN), using fatty acid and volatile compounds in meat to classify light lamb carcasses produced under three feeding diets in the Mallorquina breed.

2. Materials and methods

2.1. Sample and analysis information

The lamb samples were collected from the autochthonous Mallorquina breed on the island of Mallorca (Balearic Islands, Spain) and were raised under the traditional feeding regime of lambs from this Mediterranean area, as described by Mena and Delgado-Pertinhez (2023). According to the three feeding diets, commercial lamb categories were classified as follows: suckling lambs raised only on their mothers' milk (SL; n = 32); light lambs (referred to as "Ternasco") raised with their mothers on milk and natural pasture (TP; n = 28); and Ternasco raised with their mothers on milk, grain-based cereals and natural pasture (TC; n = 24). The lambs were slaughtered at the Palma de Mallorca slaughterhouse (Spain), following Council Regulation (EC) No 1099/2009 (2009) on the protection of animals at slaughter time.

The SL category was slaughtered immediately after weaning at the age of 36 ± 4 days, while the TP and TC categories were slaughtered at 116 ± 6 and 91 ± 5 days respectively, to obtain commercial carcasses of 6.33 ± 1.02 , 7.62 ± 1.29 and 9.57 ± 1.13 kg weight for SL, TP and TC, respectively.

After 24 h the *longissimus thoracis-lumborum* (LTL) muscle was extracted from the left side of the carcass. Portions of approximately 50 g were sliced, vacuum packed and stored at -18°C for further laboratory analysis.

The full details of the process used to identify and quantify the fatty acids and volatile compounds of the lambs' meat have been described thoroughly in our previous study (Gutiérrez-Peña et al., 2022). Fatty acid analysis was carried out following the method described by Sukhija and Palmquist (1988) and revised by Juárez et al. (2008). Initially, intramuscular fatty acid methyl esters (FAME) were extracted from approximately 1 g samples of LTL. Separation and detection of FAME were carried out using a gas chromatograph Agilent 6890N Network GS System (Agilent, Inc., Santa Clara, CA, USA) equipped with a flame ionisation detector (FID). Individual FAME were identified by comparing their retention times with those of the standards authenticated by Sigma (Sigma Chemical Co., Ltd., Poole, UK).

For the analysis of volatile compounds, samples of 20 g of LTL were previously cooked on a closed mixed griddle for approximately 3 min to a core temperature of 200°C . Following cooking, separation and detection of volatile compounds were carried out using a Thermo Scientific TRACE 1300 series (Milan, Italy) gas chromatograph (GC) equipped with a Thermo Scientific TRIPLUS RSH autosampler (Milan, Italy) for injection and coupled with an ion trap mass spectrometer (Thermo Scientific ISQ QD Single Quadrupole Mass Spectrometer). The volatile compounds were identified using an approach described by Stashenko and Martínez (2011), comparing their mass spectra with those included in NIST/EPA/NIH Mass Spectral Libraries and comparing linear retention indices (LRI) with Flavornet and PubChem databases.

2.2. Statistical analysis

The statistical analyses of the data for fatty acids and volatile compounds were performed with the Statgraphics Centurion 18 package (Royal Technologies S.A., Hudsonville, MI, USA). Homogeneity of variances was checked using Levene's test. The data was log-transformed for subsequent analyses in case it did not meet the homogeneity assumption (Levene's test; $p < 0.05$). The significant differences of fatty

acids and volatile compounds were determined by ANOVA at $p < 0.05$. Duncan's multiple range test was performed to determine which means were significantly different from others. The Kruskal-Wallis multiple comparison test was employed to identify significant differences if log-transformed variable did not meet the homogeneity assumption. Pearson's correlation analysis was performed to study the relationship among significant differences in variables.

Stepwise multiple linear regression analyses were used to calculate the F-scores for the fatty acids and volatile compounds (Lee et al., 2017). For this analysis, the same variables selected in the Pearson's correlation analyses were entered. The Durbin Watson statistic was used to measure collinearity of the model ($p > 0.05$).

2.3. Machine learning models

The classification models were evaluated by the Support Vector Machine (SVM), K-Nearest Neighbours (KNN) and Artificial Neural Network (ANN) algorithms. The machine learning algorithms were built using Python programming language, v. 3.7 (Python Software Foundation, Beaverton, USA. Available at <http://www.python.org>) and the Scikit-learn library package (Hao & Ho, 2019).

As detailed in the existing bibliography (Amirgaliev et al., 2014), various architectures of ANN exist, comprising input, hidden, and output layers, and rooted in the Multi-Layer Perceptron paradigm and diverse configuration options to determine the optimal neuron count. The model architecture was fine-tuned through parameter adjustments: the learning rate was set at 0.001, with 500 training cycles or epochs, and the hidden layers were set at 150, 100 and 50. Rectified linear units (ReLU) activation function characterized each hidden layer (Glorot et al., 2011). The model was optimized using the Adam solver function (Kingma & Ba, 2015). The output layer, representing the SL, TP, and TC lamb categories, featured three neurons. The Softmax activation function rendered the output decision as a probability distribution. To account for the risk of overfitting, dropout layers were added after the first and second hidden layers (Srivastava et al., 2014), given the relatively small size of the input instances. The SVM algorithm utilized linear kernel functions (Kotu & Deshpande, 2015). For the KNN algorithm, odd values were assessed for the K value, determining the number of nearest training records influencing the prediction of an unlabelled test record, as suggested by Kotu and Deshpande (2015). The optimal number of neighbours was evaluated for each subset by adjusting the parameter K. Uniform adjustments were applied in assigning weights for predicting the target category.

3. Results and discussion

3.1. Content of fatty acids and volatile compounds in lamb meat

To explore variations in the fatty acids profile and volatile compounds in lamb meat across three different feeding diets, ANOVA and Duncan's multiple comparative analysis were carried out. The results of this comprehensive analysis, including the mean \pm standard deviation (SD) of the fatty acid profile and volatile compounds, are presented in Tables 1 and 2, respectively. A total of 105 variables were identified, of which 33 revealed significant differences among the three feeding diets ($p < 0.05$). Among the significant variables, 16 corresponded to fatty acids (C8:0, C10:0, C11:0, C16:0, C17:1, C18:0, C18:1, 11t ATV, C18:2 n-6c, C18:3 n-3, C18:2 9c11t CLA, C22:0, C20:4 n-6, C20:5 n-3, C24:0, C22:5 n-3 and C22:6 n-3), and 17 to volatile compounds (2-butanone, 3-methylbutanal, hexanal, 3-hydroxybutan-2-one, hydroxypropan-2-one, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, acetic acid, benzaldehyde, (Z)-2-nonenal, 2-hydroxypropanoic acid, propanoic acid, 2,3-butanediol, 2-undecanone, 2-ethylhexanoic acid, octanoic acid and decanoic acid). All these statistically significant variables were selected for subsequent multivariate analysis.

The meat of suckling lambs (SL category) and pasture-fed lambs (TP

Table 1

Fatty acids (Means \pm SD, expressed in mg/100 g fresh meat) in meat of the Mallorquina lamb breed included in the study.

Variable	SL (n = 32)	TP (n = 28)	TC (n = 24)	SEM	P-value
C8:0	0.11 \pm 0.12 ^b	0.21 \pm 0.21 ^{ab}	0.24 \pm 0.10 ^a	0.018	0.001
C10:0	0.84 \pm 0.95 ^b	1.06 \pm 1.10 ^b	1.74 \pm 0.77 ^a	0.111	0.003
C11:0	0.14 \pm 0.11 ^b	0.13 \pm 0.08 ^b	0.19 \pm 0.06 ^a	0.010	0.008
C12:0	0.95 \pm 0.73	1.21 \pm 0.92	1.34 \pm 0.68	0.086	0.175
C13:0	0.07 \pm 0.07	0.06 \pm 0.03	0.08 \pm 0.05	0.006	0.349
C14:0	4.50 \pm 2.74	4.92 \pm 3.10	5.23 \pm 2.74	0.311	0.637
C14:1	0.17 \pm 0.11	0.18 \pm 0.14	0.19 \pm 0.12	0.013	0.836
C15:0	0.42 \pm 0.22	0.44 \pm 0.19	0.54 \pm 0.27	0.025	0.115
C15:1	0.07 \pm 0.07	0.06 \pm 0.04	0.34 \pm 1.24	0.073	0.242
C16:0	26.19 \pm 17.07 ^b	26.08 \pm 12.81 ^b	35.98 \pm 13.13 ^a	1.655	0.025
C16:1	1.94 \pm 1.07	1.91 \pm 0.95	2.39 \pm 1.02	0.112	0.181
C17:0	1.31 \pm 1.21	1.21 \pm 0.98	1.53 \pm 0.51	0.106	0.510
C17:1	0.48 \pm 0.19 ^b	0.48 \pm 0.19 ^b	0.72 \pm 0.31 ^a	0.028	0.000
C18:0	18.70 \pm 12.15 ^{ab}	16.10 \pm 7.62 ^b	22.25 \pm 7.56 ^a	1.067	0.018
C18:1n-9t	0.67 \pm 0.47	0.78 \pm 0.52	0.65 \pm 0.38	0.050	0.543
C18:1t11 ATV	1.43 \pm 0.71 ^b	1.81 \pm 1.12 ^{ab}	2.34 \pm 1.58 ^a	0.130	0.017
C18:1 n-9c	33.36 \pm 17.43	28.42 \pm 11.74	38.16 \pm 13.90	1.643	0.065
C18:2 n-6t	0.44 \pm 0.23	0.43 \pm 0.19	0.35 \pm 0.19	0.023	0.237
C18:2 n-6c	9.26 \pm 4.49 ^b	9.39 \pm 3.91 ^b	11.53 \pm 3.10 ^a	0.439	0.005
C18:3 n-6	0.17 \pm 0.11	0.14 \pm 0.06	0.16 \pm 0.06	0.009	0.535
C20:0	0.23 \pm 0.12	0.22 \pm 0.11	0.27 \pm 0.12	0.013	0.331
C18:3 n-3	1.45 \pm 0.52 ^a	1.17 \pm 0.42 ^b	0.79 \pm 0.55 ^c	0.061	0.000
9c11t CLA	0.68 \pm 0.31 ^{ab}	0.79 \pm 0.32 ^a	0.59 \pm 0.20 ^b	0.032	0.031
C20:1 n-9	0.22 \pm 0.12	0.20 \pm 0.09	0.24 \pm 0.09	0.011	0.470
10t12c CLA	0.10 \pm 0.05	0.12 \pm 0.08	0.08 \pm 0.05	0.007	0.066
C21:0	0.07 \pm 0.06	0.06 \pm 0.02	0.07 \pm 0.02	0.005	0.699
C20:2	0.20 \pm 0.12	0.21 \pm 0.10	0.24 \pm 0.10	0.012	0.465
C22:0	0.58 \pm 0.34 ^b	0.59 \pm 0.31 ^b	0.77 \pm 0.21 ^a	0.033	0.040
C20:3 n-6	0.19 \pm 0.13	0.15 \pm 0.07	0.23 \pm 0.15	0.014	0.081
C22:1 n-9	0.14 \pm 0.07	0.14 \pm 0.05	0.17 \pm 0.08	0.008	0.163
C20:4 n-6	5.15 \pm 2.83 ^{ab}	4.82 \pm 2.18 ^b	5.89 \pm 1.75 ^a	0.258	0.022
C20:3 n-3	0.12 \pm 0.07	0.10 \pm 0.05	0.13 \pm 0.06	0.007	0.284
C23:0	0.10 \pm 0.06	0.09 \pm 0.04	0.11 \pm 0.05	0.006	0.230
C20:5 n-3	1.25 \pm 0.54 ^a	0.90 \pm 0.38 ^b	0.60 \pm 0.36 ^c	0.056	0.000
C22:2	0.10 \pm 0.07	0.09 \pm 0.03	0.08 \pm 0.03	0.005	0.350
C24:0	0.14 \pm 0.08 ^{ab}	0.10 \pm 0.03 ^b	0.15 \pm 0.06 ^a	0.007	0.037
C24:1	0.13 \pm 0.08	0.11 \pm 0.05	0.12 \pm 0.05	0.007	0.360
C22:5 n-3	1.50 \pm 0.57 ^a	1.18 \pm 0.36 ^b	1.04 \pm 0.43 ^b	0.055	0.001
C22:6 n-3	1.16 \pm 0.73 ^a	0.68 \pm 0.29 ^b	0.55 \pm 0.33 ^c	0.062	0.000

Means with different letters (^a, ^b, ^c) in the same row are statistically different ($p < 0.05$).

SL: Suckling lambs; TP: Ternasco pasture lambs; TC: Ternasco concentrate-fed lambs.

SEM: Standard error of the mean.

category) showed higher concentrations of α -linolenic acid (C18:3 n-3), eicosapentaenoic acid (C20:5 n-3), docosahexaenoic acid (C22:6 n-3) than grain-fed lambs (TC category) (Table 1). These observations are related to the fact that the grass intake by mothers and lambs has a higher content of PUFA (such as C18:3 n-3, which is the major fatty acid in grass) compared to concentrate feed, which has a higher content of SFA than grass (Wood et al., 2008). SL showed a significantly higher concentration of C18:3 n-3 than TP ($p < 0.05$), potentially influenced by the grass-based diet of SL mothers, as described by Scerra et al. (2007). Moreover, significantly higher concentrations of C20:5 n-3, C22:5 n-3 and C22:6 n-3 were observed in meat from SL compared to TP ($p < 0.05$). These differences may be attributed to the higher concentrations of PUFA present in breast milk, particularly when the mothers have been grass-fed (Lanza et al., 2006), and an increase of elongation and desaturation of fatty acids in the mother's digestive system to convert C18:3 n-3 from grass into C20:5 n-3 C22:5 n-3 and C22:6 n-3 (Lourenço et al.,

Table 2

Volatile compounds (Means \pm SD, expressed in peak area under the identification curve $\times 10^6$) in meat of the Mallorquina lamb breed included in the study.

Variable	SL (n = 32)	TP (n = 28)	TC (n = 24)	SEM	P-value
2-butanone	42.53 \pm 3.28 ^a	33.09 \pm 2.23 ^{ab}	19.19 \pm 1.65 ^b	2.947	0.005
3-methylbutanal	363.98 \pm 27.17 ^a	435.65 \pm 38.90 ^a	171.44 \pm 18.99 ^b	34.202	0.009
Pentanal	211.12 \pm 21.65	374.89 \pm 37.42	373.80 \pm 33.04	34.449	0.069
Toluene	10.39 \pm 1.87	8.41 \pm 0.53	4.08 \pm 0.32	1.332	0.155
2,3-pentanedione	86.26 \pm 8.74	101.78 \pm 6.60	66.77 \pm 3.93	7.631	0.200
Dimethyl disulfide	12.35 \pm 1.16	12.43 \pm 1.28	7.71 \pm 0.34	1.151	0.320
Hexanal	4772.89 \pm 559.69 ^b	8559.86 \pm 792.98 ^a	9871.47 \pm 942.30 ^a	856.579	0.012
P-xylene	13.50 \pm 1.37	20.14 \pm 1.71	19.93 \pm 1.92	1.814	0.217
1-penten-3-ol	26.49 \pm 3.15	30.78 \pm 3.68	17.69 \pm 2.43	3.454	0.325
2-heptanone	27.03 \pm 1.64	35.86 \pm 4.11	28.95 \pm 3.01	3.306	0.513
Heptanal	355.00 \pm 38.99	583.08 \pm 63.48	566.90 \pm 92.18	71.960	0.333
2-pentylfuran	74.60 \pm 10.05	130.59 \pm 15.48	101.23 \pm 12.20	13.911	0.239
1-pentanol	123.37 \pm 16.19	155.60 \pm 15.35	197.56 \pm 23.72	20.107	0.333
Methylpyrazine	23.84 \pm 1.60	21.94 \pm 2.31	18.75 \pm 2.58	2.333	0.682
2-octanone	5.56 \pm 0.75	4.93 \pm 0.47	3.60 \pm 0.39	0.624	0.448
Octanal	185.07 \pm 25.82	268.98 \pm 31.82	318.66 \pm 38.05	34.686	0.282
3-hydroxybutan-2-one	202.53 \pm 21.68 ^a	57.25 \pm 9.34 ^b	100.92 \pm 6.26 ^b	17.427	0.004
1-octen-3-one	14.00 \pm 1.42	22.20 \pm 2.03	21.90 \pm 2.41	2.143	0.190
Hydroxypropan-2-one	12.16 \pm 0.49 ^a	11.74 \pm 0.57 ^a	8.53 \pm 0.71 ^b	0.652	0.001
2,5-dimethylpyrazine	109.90 \pm 2.54 ^c	157.76 \pm 4.19 ^a	134.87 \pm 4.11 ^b	4.486	0.000
2,3-octanedione	645.78 \pm 43.50	722.50 \pm 52.85	825.69 \pm 80.33	64.152	0.532
2,6-dimethylpyrazine	42.27 \pm 2.06 ^b	53.66 \pm 1.87 ^a	41.86 \pm 2.18 ^b	2.275	0.000
2,3-dimethylpyrazine	16.89 \pm 0.71	24.34 \pm 2.08	19.86 \pm 1.18	1.575	0.135
1-hexanol	607.73 \pm 173.22	315.51 \pm 83.96	145.61 \pm 21.32	129.093	0.335
2-ethyl-6-methylpyrazine	12.67 \pm 1.08	12.03 \pm 0.99	8.28 \pm 1.23	1.199	0.301
Nonanone	9.58 \pm 0.40	10.45 \pm 0.78	8.34 \pm 0.80	0.727	0.529
Nonanal	547.25 \pm 66.50	557.90 \pm 58.13	712.01 \pm 92.01	78.297	0.658
Trimethylpyrazine	81.73 \pm 2.96	80.76 \pm 4.62	74.31 \pm 5.13	4.577	0.790
2-ethyl-3-methylpyrazine	16.19 \pm 1.39	19.77 \pm 2.04	23.52 \pm 2.49	2.153	0.391
Octenal	9.15 \pm 0.74	14.32 \pm 1.12	13.53 \pm 1.51	1.246	0.168
3-ethyl-2,5-dimethylpyrazine	27.89 \pm 1.78	27.33 \pm 2.27	22.53 \pm 2.44	2.330	0.616
1-octen-3-ol	201.25 \pm 28.88	243.41 \pm 22.64	318.04 \pm 55.54	40.174	0.505
Heptanol	26.40 \pm 4.67	29.10 \pm 3.32	32.76 \pm 4.97	4.709	0.865
2-ethyl-3,5-dimethylpyrazine	10.28 \pm 0.73	10.65 \pm 0.91	9.11 \pm 1.11	0.984	0.820
2-propanone	5.55 \pm 0.32	5.57 \pm 0.40	3.90 \pm 0.36	0.393	0.163

Table 2 (continued)

Variable	SL (n = 32)	TP (n = 28)	TC (n = 24)	SEM	P-value
Furfural	2.62 \pm 0.15	3.17 \pm 0.16	2.84 \pm 0.20	0.180	0.446
Acetic acid	43.43 \pm 1.65 ^a	29.71 \pm 1.70 ^b	34.73 \pm 2.39 ^{ab}	2.150	0.022
2-ethylhexan-1-ol	26.17 \pm 2.25	30.04 \pm 2.47	23.29 \pm 1.50	2.334	0.525
2-decanone	6.08 \pm 0.43	5.90 \pm 0.53	4.22 \pm 0.50	0.531	0.317
3,5-diethyl-2-methylpyrazine	12.34 \pm 2.41	12.08 \pm 0.95	8.03 \pm 1.02	1.819	0.587
Decanal	7.24 \pm 0.58	8.99 \pm 0.73	7.58 \pm 0.56	0.685	0.539
2-acetylfuran	13.56 \pm 5.31	3.62 \pm 0.28	4.44 \pm 0.33	3.589	0.437
Pyrrrole	6.37 \pm 0.34	8.99 \pm 0.78	6.16 \pm 0.55	0.636	0.133
Benzaldehyde	67.65 \pm 2.79 ^b	77.44 \pm 3.04 ^a	49.30 \pm 2.26 ^c	3.194	0.001
(Z)-2-nonenal	9.50 \pm 0.62 ^b	15.19 \pm 1.33 ^a	10.60 \pm 1.32 ^b	1.230	0.044
2-hydroxypropanoic acid	4.26 \pm 0.26 ^a	2.50 \pm 0.20 ^b	3.21 \pm 0.28 ^b	0.275	0.003
Octanol	25.58 \pm 2.84	28.50 \pm 3.11	35.71 \pm 5.34	4.120	0.608
Propanoic acid	2.40 \pm 0.13 ^a	1.45 \pm 0.09 ^b	2.44 \pm 0.18 ^a	0.156	0.013
3,5-octadien-2-one	4.20 \pm 0.45	6.59 \pm 0.74	3.66 \pm 0.31	0.594	0.236
3-methylpyrrrole	2.76 \pm 0.22	2.09 \pm 0.13	7.74 \pm 1.66	1.005	0.777
2,3-butanediol	31.09 \pm 2.84 ^a	5.38 \pm 0.56 ^b	34.98 \pm 2.74 ^a	2.866	0.000
2-undecanone	1.60 \pm 0.10 ^b	2.60 \pm 0.14 ^a	1.81 \pm 0.12 ^b	0.138	0.006
(E)-2-octen-1-ol	11.85 \pm 1.31	15.56 \pm 1.37	20.21 \pm 4.02	2.636	0.446
Butyrolactone	32.77 \pm 2.03	30.27 \pm 1.85	28.63 \pm 1.82	2.071	0.716
Benzeneacetaldehyde	9.95 \pm 1.17	7.93 \pm 0.58	6.16 \pm 0.58	0.939	0.262
2-furanmethanol	5.41 \pm 0.62	3.93 \pm 0.28	3.72 \pm 0.31	0.491	0.294
3-methylbutanoic acid	5.72 \pm 0.50	7.22 \pm 0.72	7.17 \pm 1.09	0.845	0.703
2(E),4(Z)-undecadienal	12.46 \pm 1.97	10.18 \pm 0.41	10.01 \pm 0.59	1.389	0.716
2(E),4(Z)-dodecadienal	4.84 \pm 0.43	6.22 \pm 0.54	6.43 \pm 0.69	0.597	0.487
Hexanoic acid	11.52 \pm 0.81	15.51 \pm 1.69	14.00 \pm 1.03	1.334	0.447
Dimethyl sulfone	8.05 \pm 0.87	9.83 \pm 1.25	6.25 \pm 0.64	1.053	0.415
2-ethylhexanoic acid	0.50 \pm 0.01 ^b	0.56 \pm 0.01 ^a	0.81 \pm 0.07 ^a	0.041	0.000
Heptanoic acid	1.41 \pm 0.06	1.58 \pm 0.10	2.24 \pm 0.15	0.120	0.448
2-acetylpyrrrole	12.87 \pm 0.87	11.31 \pm 0.49	8.94 \pm 0.62	0.765	0.116
Octanoic acid	2.10 \pm 0.11 ^b	2.48 \pm 0.15 ^{ab}	3.81 \pm 0.25 ^a	0.202	0.023
Decanoic acid	4.54 \pm 0.41 ^b	6.20 \pm 0.57 ^b	10.51 \pm 0.76 ^a	0.683	0.000

Means with different letters (^a, ^b, ^c) in the same row are statistically different ($p < 0.05$).

SL: Suckling lambs; TP: Ternasco pasture lambs; TC: Ternasco concentrate-fed lambs.

SEM: Standard error of the mean.

2007). TC category showed the highest concentrations of caprylic acid (C8:0), capric acid (C10:0), undecanoic acid (C11:0), palmitic acid (C16:0), stearic acid (C18:0), docosanoic acid (C22:0), tetracosanoic acid (C24:0) and heptadecenoic acid (C17:1) (Table 1). These findings are in line with previous studies by Fisher et al. (2000) and Nuernberg

et al. (2008), which compared lambs fed on grass versus grain.

In addition to the fatty acid profile, volatile compounds have been reported as effective biomarkers for classifying lamb meat according to the feeding diet (Vasta et al., 2012). Among the volatile compounds exhibiting significant differences ($p < 0.05$) (Table 2), we identified four aldehydes (3-methylbutanal, hexanal, benzaldehyde, and (Z)-2-nonenal), four ketones (2-butanone, 3-hydroxybutan-2-one, hydroxypropan-2-one, and 2-undecanone), two pyrazines (2,

5-dimethylpyrazine and 2,6-dimethylpyrazine), one alcohol (2,3-butanediol), and six carboxylic acids (acetic acid, 2-hydroxypropanoic acid, propanoic acid, 2-ethylhexanoic acid, octanoic acid, and decanoic acid). While almost all of these compounds were formed from lipid oxidation, the only Maillard-derived compounds detected with significant differences among the feeding regimes of the lambs were pyrazines, 3-hydroxybutan-2-one, hydroxypropan-2-one, and 3-methylbutanal (Elmore et al., 2005).

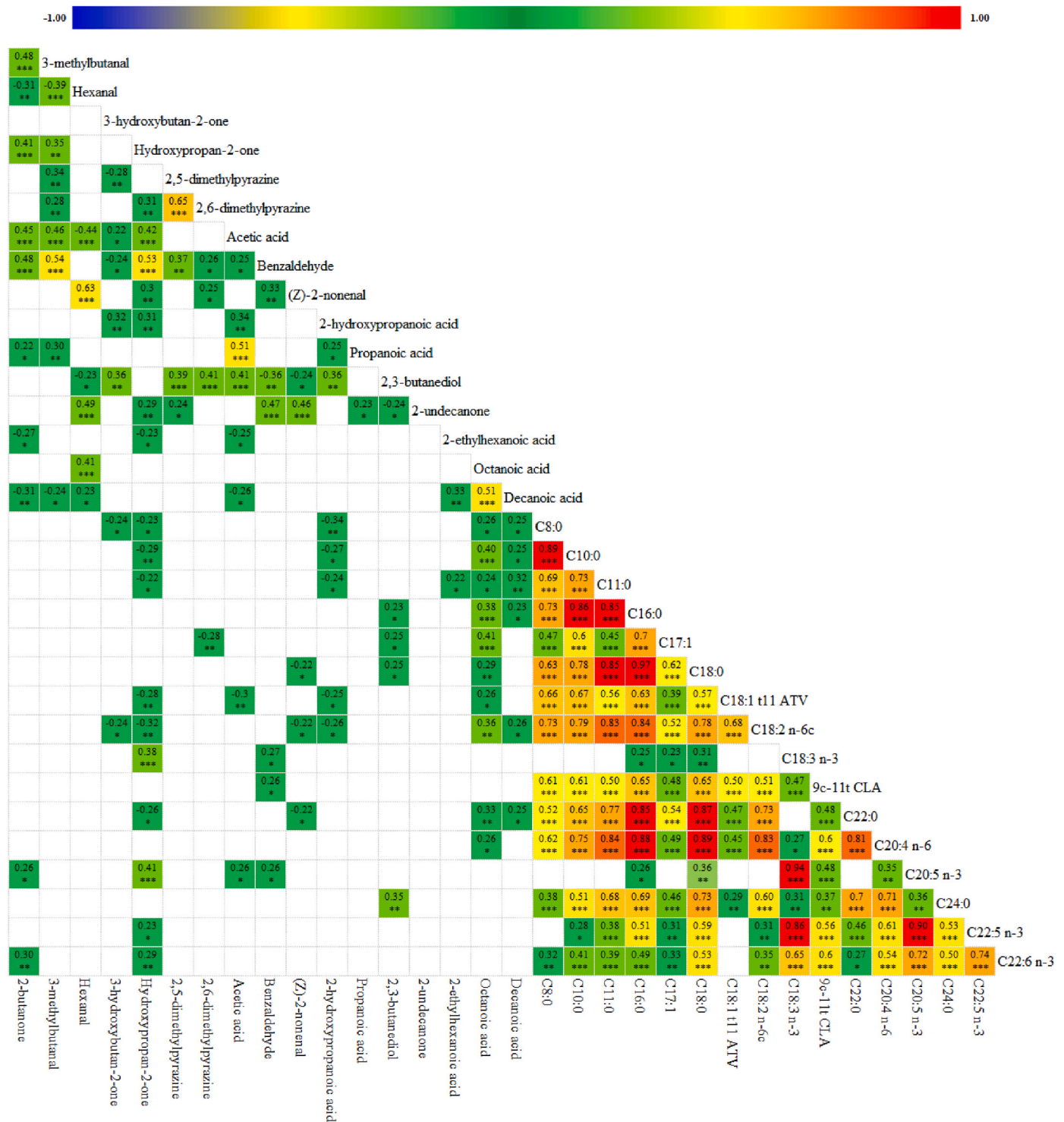


Fig. 1. Correlation matrix plot of significant variables selected. Colours of squares represent magnitude of correlations. Blank squares indicate non-significant correlations ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Aldehydes and ketones are considered to be significant odorants in meat that are affected by animal diet (Resconi et al., 2010). In fact, carbonyl compounds derived from lipid oxidation may vary depending on the feeding regime (Vasta & Priolo, 2006). On the other hand, Young et al. (2003) and Priolo et al. (2004) identified specific volatile compounds in neutral lipids from subcutaneous and perirenal fat deposits which differentiate the dietary origin of heavy lambs. These works identified skatole as a compound associated to grass intake and found branched chain fatty acids to be related to concentrated feed intake. However, Vasta et al. (2007) reported that these differences in intramuscular fat are not so apparent because skatole and branched-chain fatty acids are mainly located in the reserve fat depots. Our results regarding volatile compound profiles in the intramuscular fat of light lambs align with the observations reported by Vasta et al. (2007), who noted an absence of skatole and low branched-chain fatty acid content in intramuscular fat.

Considering these observations, where specific biomarkers such as skatole and branched-chain fatty acids in intramuscular fat are not evident for differentiating feeding regimes in light lambs, exploring new patterns that link fatty acids and volatile compounds could help to classify light lamb categories effectively, and machine learning could be a valid tool to achieve this (Sabilla et al., 2020).

3.2. Multivariate analysis and variable reduction steps

Multivariate statistical analysis was used to reduce analysed variables, and machine learning algorithms were utilized to classify the lamb carcass categories. In order to prevent variables with larger scales from dominating the analysis for identifying the category of lamb and the effect on the analysis of different units in the selected variables, the database was standardized to have 0 mean and 1 standard deviation (Maione et al., 2016). To address any potential statistical bias in the multiple regression analysis, a Pearson's correlation analysis was conducted (Krackhardt, 1988). The correlation matrix plot is shown in Fig. 1. The significant correlations identified ($p < 0.05$) ranged between -0.44 and 0.97 . High correlations were observed between C16:0 and C18:0 (0.97), C18:3 n-3 and C20:5 n-3 (0.94), C20:5 n-3 and C22:5 n-3 (0.90), C8:0 and C:10 (0.89), C18:0 and C20:4 n-6 (0.89), C16:0 and C20:4 n-6 (0.88), C18:0 and C22:0 (0.87), C10:0 and C16:0 (0.86), C18:3 n-3 and C22:5 n-3 (0.86), C11 and C18:0 (0.85), and C16:0 and C22:0 (0.85). The inclusion of highly correlated compounds in the classification model was contingent on the F-score that was subsequently calculated.

To select the variables for the classification model, a stepwise forward variable importance analysis was conducted by calculating the F-

score in a multiple regression model. Following the calculation of the F-score, the variables were arranged from highest to lowest F-score, and strongly correlated variables ($p < 0.05$; Pearson $> |0.85|$) were removed from the model. Among the pairs of highly-correlated variables, those with the highest F-score were selected. Based on these criteria, the fatty acids C18:3 n-3, C22:5 n-3, C10:0, C24:0, C16:0 and C18:0 were removed from the model. Finally, variables with F-score > 3 were selected to create the classification model. With these variable selection criteria, multiple regression modelling was run with the 14 selected variables shown in Fig. 2. The analysis showed significant results ($p < 0.05$) with $R^2 = 0.85$, indicating a good fit for the model, as reported by Wang and Jain (2003). As an additional verification measure, the Durbin-Watson bounds test was applied to assess autocorrelation coefficients for positive values, with a significance level of $p > 0.05$ (L'Esperance & Taylor, 1975).

The F-score values assigned to each variable selected are shown in Fig. 2, with the variables listed according to the F-score value. The C17:1, C20:5 n-3, 3-methylbutanal and 2,5-dimethylpyrazine variables have the largest scores compared with the other variables. In fact, C17:1 and C20:5 n-3, among other fatty acids, have both been reported as having high classifying ability in lambs, especially to classify their feeding diets (Díaz et al., 2005; Velasco et al., 2004). While Vasilev et al. (2020) identified C18:3 n-3 and C20:5 n-3 as fatty acids with major importance in the differentiation of lamb meat, in our research, we excluded C18:3 n-3 from the classification model due to its high collinearity (0.94) with C20:5 n-3. This finding supports our selection of C20:5 n-3 in the classification model. Moreover, 2,5-dimethylpyrazine and 3-methylbutanal were included in the model as compounds with high discriminate power in lamb meat based on the feeding diet, as reported by both Gkarane et al. (2019) and Frank et al. (2016).

In addition to the discriminant potential exhibited by compounds with high F-scores, it has been observed that C17:1 is a metabolic byproduct resulting from the desaturation of C17:0 (Pimentel et al., 2021), potentially linked to the formation of volatile compounds with off-flavour notes (Song et al., 2017). Despite its minor presence in the lamb's fatty acid profile, the association with concentrate feed intake revealed in the results suggests that it may be a promising fatty acid for future research concerning meat quality.

Other compounds with high F-scores that have an impact on meat quality are C20:5 n-3 and C22:6 n-3. Their increased presence in the meat is associated with the elongation and desaturation of dietary C18:3 n-3 originating from pasture (Díaz et al., 2011). This relationship is consistent with our results, where positive correlations were obtained among C20:5 n-3, C22:6 n-3 and C18:3 n-3. Regarding the flavour provided by these fatty acids, the importance they have in meat flavour

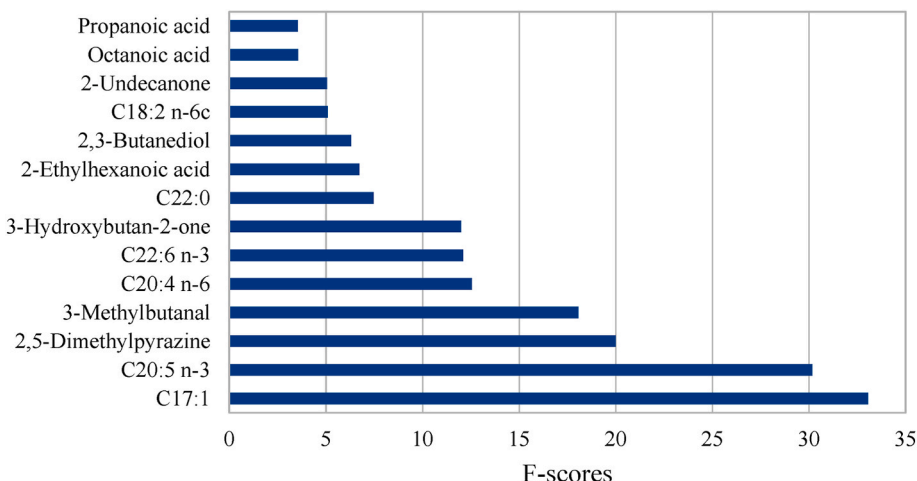


Fig. 2. Relative variable importance based on calculated F-scores.

is well known. While C18:3 n-3 fatty acid is positively correlated with lamb flavour quality (Sañudo et al., 2000), high n-3 PUFA content in meat is related to a 'rancid flavour' due to high lipid oxidation (Elmore et al., 2005). However, in grass-raised lambs, antioxidants from the pasture prevent the rancid flavour, thereby improving the meat quality (Luciano et al., 2013; Luo et al., 2019).

3.3. Machine learning algorithm for classifying lamb carcasses

After identifying the relative importance of the selected variables, we created variable subsets for use in each machine learning algorithm for classification analysis. Each subset was generated with the top N variables that exhibited the highest F-score values, where $N = \{1, 2, \dots, 14\}$, with subset #1 including the top-rated variable, subset #2 the two top-rated variables, and so on. The final subset, #14, contained all the selected variables (Table 3). We used this method to reduce the dimensionality and to gain insights into the behaviour of the classification models when the variables were added to the training models. Once the subset had been introduced into the algorithm, a holdout cross-validation process was used to split the data set randomly into a training set (75%) and a validation set (25%). Given the imbalance in the data, a stratify function was applied using the same proportion in each class, as recommended by Prati et al. (2015).

Table 3 shows the variable subsets generated, and the performance achieved by the SVM, KNN and ANN algorithms. We observed a different pattern in how the three algorithms performed for each subset of variables. SVM reached its peak performance (0.86) at subset #7 but failed to show further improvement. In contrast, KNN tended to improve as top-rated variables were added, reaching its highest performance (0.98) with subset #13. Meanwhile, ANN achieved its maximum performance (0.98) at both subset #7 and #14. Thus, we found that the best-performing option was a combination of subset #7 and the ANN algorithm, as it achieved maximum accuracy with the lowest number of variables, and the subset formed by C17:1, C20:5 n-3, 2,5-dimethylpyrazine, 3-methylbutanal, C20:4 n-6, C22:6 n-3, and 3-hydroxybutan-2-one appeared to be the optimal choice for achieving the best classification of the studied groups. In this way, higher concentrations of C17:1 and C20:4 n-6 seem to be related to the intake of concentrated feed,

Table 3

Accuracy values achieved by the classification algorithms using different subsets of variables.

Subset	Variables ^a	Accuracy		
		SVM	KNN ^b	ANN
#1	1	0.46	0.45 (4)	0.48
#2	1,2	0.47	0.62 (4)	0.67
#3	1,2,3	0.74	0.71 (6)	0.71
#4	1,2,3,4	0.77	0.90 (3)	0.90
#5	1,2,3,4,5	0.77	0.71 (3)	0.81
#6	1,2,3,4,5,6	0.85	0.88 (4)	0.88
#7	1,2,3,4,5,6,7	0.86	0.86 (4)	0.98
#8	1,2,3,4,5,6,7,8	0.86	0.86 (4)	0.86
#9	1,2,3,4,5,6,7,8,9	0.86	0.90 (4)	0.85
#10	1,2,3,4,5,6,7,8,9,10	0.86	0.90 (7)	0.87
#11	1,2,3,4,5,6,7,8,9,10,11	0.81	0.95 (6)	0.90
#12	1,2,3,4,5,6,7,8,9,10,11,12	0.86	0.90 (4)	0.97
#13	1,2,3,4,5,6,7,8,9,10,11,12,13	0.80	0.98 (4)	0.92
#14	1,2,3,4,5,6,7,8,9,10,11,12,13,14	0.82	0.86 (3)	0.98

SVM: Support Vector Machine; KNN: K-Nearest Neighbours; ANN: Artificial Neural Network.

Values in bold are the best accuracy achieved for each algorithm.

^a 1 = C17:1; 2 = C20:5 n-3; 3 = 2,5-dimethylpyrazine; 4 = 3-methylbutanal; 5 = C20:4 n-6; 6 = C22:6 n-3; 7 = 3-hydroxybutan-2-one; 8 = C22:0; 9 = 2-ethylhexanoic acid; 10 = 2,3-butanediol; 11 = C18:2 n-6c; 12 = 2-undecanone; 13 = Octanoic acid; 14 = Propanoic acid.

^b In parentheses: number of neighbours used. The estimation is based on the best precision obtained among the first thirty K values, simulated during the training of the model.

while higher concentrations of 2,5-dimethylpyrazine and 3-methylbutanal are associated with grass feeding. Finally, the biomarker specifically associated with milk intake is 3-hydroxybutan-2-one, along with higher concentrations of C20:5 n-3, C22:6 n-3 and 3-methylbutanal. Among the biomarkers identified as having the greatest discriminatory power, C20:5 n-3, C22:6 n-3 and 2,5-dimethylpyrazine showed significant differences between the three lamb categories, as shown in Tables 1 and 2

We made an interesting observation regarding the performance of subset #4, which includes the biomarkers C17:1, C20:5 n-3, 2,5-dimethylpyrazine, and 3-methylbutanal. This subset proved suitable for classifying the feeding diet, with a fair accuracy rate of 0.90 using the KNN and ANN algorithms. Based on the results obtained, we suggest that fat is one of the main classifiers of lambs according to their feeding diet (Cabiddu et al., 2022). Specifically, in our study, C17:1 fatty acid seemed to help to classify the lambs that were raised with concentrate feed efficiently (TC group), while C20:5 n-3 fatty acid was associated with pasture feed and contributed to the differentiation of the three lamb categories studied (SL, TP, and TC). Moreover, 2,5-dimethylpyrazine and 3-methylbutanal, which are compounds derived from the Maillard reaction and are related to the concentration of sugars and amino compounds in the muscle (Mottram, 1998), could be proposed as a potent complement to the classifying power of fat.

On analysing subset #7, it was found to consist of 4 fatty acids and 3 volatile compounds. This indicates that fatty acids are more important as classifying variables when considering subsets with fewer variables. Meanwhile, the analysis of subsets #13 and #14 revealed that as the number of variables increases, the importance of the volatile compounds in carcass classification gradually increases with the addition of variables, with subsets #13 and #14 consisting of 6 fatty acids, and 7 and 8 volatile compounds, respectively.

Table 4 shows the confusion matrix for the best-performing subset with each algorithm. SVM is shown to be the least effective of the algorithms used, with TP mainly misclassified as either SL or TC. Despite

Table 4

Confusion matrices obtained for lamb carcass categories based on different feeding diets classified by SVM, KNN and ANN algorithms with the best model performance.

	Lamb category	Predicted group membership for SVM with subset #7			Accuracy
		Predicted class			
		SL	TP	TC	
Observed class	SL	7	1	0	0.88
	TP	1	6	0	0.86
	TC	0	1	5	0.83
Overall accuracy					0.86
	Lamb category	Predicted group membership for KNN with subset #13			Accuracy
		Predicted class			
		SL	TP	TC	
Observed class	SL	8	0	0	1.00
	TP	1	6	0	0.86
	TC	0	0	6	1.00
Overall accuracy					0.98
	Lamb category	Predicted group membership for ANN with subset #7			Accuracy
		Predicted class			
		SL	TP	TC	
Observed class	SL	8	0	0	1.00
	TP	1	6	0	0.86
	TC	0	0	6	1.00
Overall accuracy					0.98

SL: Suckling lambs; TP: Ternasco pasture lambs; TC: Ternasco concentrate-fed lambs.

Variables included in each subset are shown in Table 3.

SVM being recognized in many studies as an outstanding classification algorithm (Maione et al., 2016; Qi et al., 2021), in certain cases, the decision boundary of an SVM classifier may skew towards the minority class for imbalanced data, leading to a high misclassification rate for minority samples (Imam et al., 2006). Additionally, the set size also affects the performance of the algorithm (Xu et al., 2021). For this reason, the nature of the data may have resulted in a lower performance of SVM compared to the other two algorithms used to achieve high classification accuracy.

The KNN and ANN algorithms showed the best classificatory performance (0.98). KNN is a non-parametric linear classification method used to categorize samples according to the majority of their k-neighbourhood members in the training set. The optimal efficiency of discrimination depends on parameter k, which is determined by a cross-validation method (Han et al., 2020). In our study, the higher correct classification with subset #13 was achieved when $k = 4$. Only in one case did the KNN and ANN algorithms misclassify the TP class for SL (Table 4).

Similar results to those of KNN were observed with ANN, although with fewer variables in the subset. An ANN is a mathematical model for information processing that mimics the synaptic connections of the brain, exhibiting nonlinear dynamic properties (Qi et al., 2021). The effectiveness of neural networks in food traceability has been reported in several studies, such as those conducted by González-Domínguez et al. (2020) in Iberian hams or Doyle et al. (2023) in beef meat, among others. While both SVM and ANN map data in a high-dimensional space and then perform regression or classification, ANN outperforms SVM because ANN actively seeks effective mapping, whereas SVM adopts a passive approach to mapping (Qi et al., 2021).

4. Conclusions

Our results show that the classification of light lamb carcasses according to feeding diet using several fatty acids and volatile compounds biomarkers is possible with a high level of performance. Among the 14 significant biomarkers selected after using the multivariate reduction technique, C17:1, C20:5 n-3, 2,5-dimethylpyrazine, 3-methylbutanal, C20:4 n-6, C22:6 n-3, and 3-hydroxybutan-2-one are the ones with the greatest discriminating power for the samples. This result has been obtained with the ANN algorithm, which in our case is the best algorithm using the seven major discriminating biomarkers. The KNN algorithm also achieved the same overall accuracy as ANN, but increased the number of variables to classify the carcass category according to the origin feeding regime. For this reason, it can be considered a useful alternative to ANN.

From a nutritional perspective, due to their desirable fatty acid content, the consumption of meat from lambs raised on milk and grass offers health advantages for consumers over meat from lambs raised using concentrate. Furthermore, a selection of fatty acids and volatile compounds of meat can be proposed as biomarkers for light lamb carcass classification using machine learning-based classification techniques.

In this research, we successfully developed a promising classification model for light lamb carcasses despite having only a small dataset. However, future works in this area should be undertaken to improve the robustness of classification models for lamb carcasses and other livestock species. This methodology could be combined with non-destructive techniques to ensure the traceability of meat production and to provide information for consumers who are concerned about the origin of the food they consume. Further research, including the generation of databases related to animal feeding systems, organoleptic quality of meat, geographical origin of animals, and genetics, among others, will greatly enhance the applicability and effectiveness of machine learning techniques in developing traceability systems for the food chain.

CRedit authorship contribution statement

M. García-Infante: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **P. Castro-Valdecantos:** Writing – review & editing, Data curation. **M. Delgado-Pertíñez:** Writing – review & editing, Methodology, Investigation, Formal analysis. **A. Teixeira:** Validation, Supervision. **J.L. Guzmán:** Methodology, Investigation, Formal analysis. **A. Horcada:** Writing – review & editing, Writing – original draft, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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