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# Principal component and multivariate factor analysis of detailed sheep milk fatty acid profile

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## ABSTRACT

Fatty acid (FA) profile is one of the most important aspects of the nutritional properties of milk. The FA content in milk is affected by several factors such as diet, physiology, environment, and genetics. Recently, principal component analysis (PCA) and multivariate factor analysis (MFA) have been used to summarize the complex correlation pattern of the milk FA profile by extracting a reduced number of new variables. In this work, the milk FA profile of a sample of 993 Sarda breed ewes was analyzed with PCA and MFA to compare the ability of these 2 multivariate statistical techniques in investigating the possible existence of latent substructures, and in studying the influence of physiological and environmental effects on the new extracted variables. Individual scores of PCA and MFA were analyzed with a mixed model that included the fixed effects of parity, days in milking, lambing month, number of lambs born, altitude of flock location, and the random effect of flock nested within altitude. Both techniques detected the same number of latent variables (9) explaining 80% of the total variance. In general, PCA structures were difficult to interpret, with only 4 principal components being associated with a clear meaning. Principal component 1 in particular was the easiest to interpret and agreed with the interpretation of the first factor, with both being associated with the FA of mammary origin. On the other hand, MFA was able to identify a clear structure for all the extracted latent variables, confirming the ability of this technique to group FA according to their function or metabolic origin. Key pathways of the milk FA metabolism were identified as mammary gland de novo synthesis, ruminal biohydrogenation, desaturation performed by stearoyl-coenzyme A desaturase enzyme, and rumen microbial activity, confirming previous findings in sheep and in other species. In general, the new extracted variables were mainly affected by physiological factors as days in milk, parity, and lambing month; the number of lambs born had no effect on the new variables, and altitude influenced only one principal component and factor. Both techniques were able to summarize a larger amount of the original variance into a reduced number of variables. Moreover, factor analysis confirmed its ability to identify latent common factors clearly related to FA metabolic pathways.

**Key words:** fatty acids, principal components, factor analysis, milk

## INTRODUCTION

The interest by the scientific community and consumers in the nutritional and health-related properties of milk and dairy products has increased over the last decades. Strategies for improving the milk content of some categories of fatty acids (**FA**) considered beneficial for human health, such as PUFA and CLA, have been developed. Most of them rely on feeding management (Dewhurst et al., 2006; Toral et al., 2010; Nudda et al., 2014) with diet being one of the most important factors affecting milk FA profile (Nudda et al., 2014). However, other factors such as physiology (De La Fuente et al., 2009), environment (Sevi et al., 2002), and genetics (Carta et al., 2008; Correddu et al., 2019) can affect milk FA composition. Thus, for example, genomic strategies to improve milk FA profile have been also proposed (Cesarani et al., 2019; Gebreyesus et al., 2019).

The elucidation of FA metabolic pathways and the knowledge of factors affecting their regulation are of great interest for improving milk nutritional properties. In particular, the complex phenotypic and genetic correlation pattern existing among individual milk FA hampers the modification of FA profile via feeding and genetic strategies (Cecchinato et al., 2019). Dimension-reduction multivariate statistical methods have been

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suggested for investigating such a complex correlation network. In particular, principal component analysis (**PCA**; Fievez et al., 2003; Kadegowda et al., 2008) and multivariate factor analysis (**MFA**; Mele et al., 2016; Correddu et al., 2017; Palombo et al., 2020) have been used to highlight common metabolic pathways of FA in ruminant species.

Being both based on the factorization of the covariance or correlation matrix, and on the representation of the multivariate system with a lower number of new variables, PCA and MFA appear somewhat similar. However, the way the factorization is carried out differs between the 2 techniques. Principal component analysis is a model-free approach aimed at compressing the variance of the system in a smaller number of new variables. On the other hand, MFA starts from a model of the covariance structure of the multivariate system. In particular, the factor model assumes that the covariance of a system could be partitioned in a component shared by all the variables (communality) plus a component specific of each variable (uniqueness). Multivariate factor analysis aims at investigating the covariance structure of the system by identifying a set of common latent variables (factors) that generate the quota of shared covariance among the original variables (Morrison et al., 1976; Krzanowski, 2000).

Principal component analysis of cattle milk FA composition was able to assess the relationship between individual milk FA and diet-induced milk fat depression (Kadegowda et al., 2008), and to investigate metabolic relationships among milk FA and to describe their origin (Fievez et al., 2003). Principal component analysis has been also used to analyze meat FA profile to differentiate lamb meat according to their origin (Díaz et al., 2005), and to study the relationship between quality traits of carcass and meat of light lamb (Cañeque et al., 2014). Multivariate factor analysis was successfully exploited to elucidate relationship between milk FA in dairy cows (Conte et al., 2016; Mele et al., 2016), sheep (Palombo et al., 2020), and buffalo (Correddu et al., 2017).

The use of the 2 methods on the same data may provide different and complementary results. In a study of cattle lactation curve traits, for example, PCA was able to extract from the correlation matrix of test day records 2 new variables related to the whole lactation and to the shape of the lactation curve, respectively. On the same data, MFA generates 2 latent factors related to the first and the second part of lactation, respectively (Macciotta et al., 2006).

The aim of this work was to compare results of MFA and PCA in the analysis of milk FA profile in sheep to assess their ability to investigate the complex correlation pattern that exists among these variables.

## MATERIALS AND METHODS

### Animals and Milk Samples

The study was carried out on individual milk samples of 993 Sarda dairy ewes farmed in 48 flocks located in the island of Sardinia (Italy). Individual milk samples (one per sheep) were collected from April to July 2014, during the morning milking, by the Provincial Association of Animal Breeders. The FA profile of the milk samples was measured using GC as previously described (Correddu et al., 2017).

### Statistical Analysis

Data for a total of 49 individual FA (expressed as g/100 g of total FA) were analyzed with PCA and MFA using SAS PRINCOMP and FACTOR procedures, respectively (SAS Institute Inc., Cary, NC). The number of principal components (**PC**) to retain was defined according to the amount of explained variance (≥80%). In MFA, the number of factors to be extracted was based on their eigenvalue (>1; Morrison et al., 1976), on their readability in terms of relationships with the original variables and biological meaning, and on the amount of explained variance. Factor interpretation was improved through a VARIMAX rotation. The VARIMAX is an orthogonal rotation, based on the maximization of the sum of squares of factor loadings (Kaiser, 1958; Forina et al., 1989).

Scores of PC and factors were then calculated for each of the 993 ewes and treated as new phenotypes.

The PC and factor scores were analyzed with the following mixed linear model:

$$y_{ijklmno} = \mu + PAR_j + DIM_k + LM_l + LB_m + ALT_n + f(ALT)_o + e_{ijklmno},$$

where $y_{ijklmno}$ was the principal component or factor score; $\mu$ was the overall mean; $PAR$ is the fixed effect of the $j$th parity class (8 classes from 1 to >7); $DIM$ is the fixed effect the $k$th days in milking interval (5 intervals: <110, 110 to 140, 141 to 170, 171 to 200, and >200); $LM$ is the fixed effect of the $l$th class of lambing month (1: January; 2: February and March; 3: October and November; 4: December); $LB$ is the fixed effect of the $m$th number of lambs born (2 classes: single and multiple birth); $ALT$ is the fixed effect of the $n$th altitude of location of flocks (mountain >500 m above sea level, hill ≤500 and ≥200 m above sea level, and plain <200 m above sea level). Finally, $f(ALT)$ is the random effect of the $o$th flock nested within the nth class of altitude, and $e_{ijklmno}$ is the residual term. Covariance matrices for

random effects were $\mathbf{I}\sigma^2_{f(ALT)}$ and $\mathbf{I}\sigma^2_e$, where $\mathbf{I}$ is an identity matrix and $\sigma^2_{f(ALT)}$ and $\sigma^2_e$ are the variance components associated with the effect of the flock nested within the altitude and with the residuals, respectively.

The contribution of the flock nested within the altitude factor ($r^2_{f(ALT)}$) was calculated as

$$r^2 f(ALT) = \frac{\sigma^2_{f(ALT)}}{\sigma^2_{f(ALT)} + \sigma^2_e}.$$

## RESULTS AND DISCUSSION

Descriptive statistics of detailed milk FA composition of the 993 samples of sheep milk are reported in Supplemental Table S1 (https://doi.org/10.3168/jds.2020-19087).

### Principal Component Analysis

Nine out of 49 PC were able to explain about 80% of the total variance of the system (Table 1). The variance explained ranged from about 25% for PC1 to about 3% for PC9, respectively. The PC scores are often used in dispersion plots to highlight possible clustering or trends in the observations. In the present work, no clear clustering of observations has been detected in the space of the first 2 PC, even though an overlapped stratification according to parity (Figure 1a) or DIM class (Figure 1b) could be appreciated. However, when the number of carbons and the level of unsaturation were used to highlight possible effects on the PC loadings, a partial clustering was observed. In Figure 2 the PC1 and PC2 scores were classified based on 4 classes of quartile distribution of the mean carbon chain length and mean of unsaturation level, calculated according to Kaylegian et al. (2009). An effect of carbon chain length classes can be observed on the PC1 scores (Figure 2a) that is able to separate, even if not in a clear manner, animals with milk FA profile characterized by different means of carbon chain length. Animals belonging to the class with the lower levels of carbon chain length had lower scores PC1; as the levels of carbon chain length increased, the animals exhibited increasing PC1 scores. The effect of the mean of unsaturation level was less evident in separating animals across the PC2 scores (Figure 2b).

The analysis of eigenvector structure is a way for assigning a meaning to the extracted PC in terms of relationship with the original variables. In the present study, the interpretation of the extracted PC on the basis of their eigenvectors (Table 1) was rather difficult. The correlation circles (Figure 3) are often used to assess the meaning of the PC, by looking for specific clusters among the original variables in the new space of the PC, and to interpret the relationship between the variables and the extracted PC. Indeed, in Figure 3 the considered FA are represented by their correlation with the first 2 extracted PC. These correlations are approximated by the angle between the vectors, with small angles indicating positive correlations and angles close to 180 degrees indicating negative correlations. However, in the present work no clear clustering was observed among original variables based on the PC loadings (as an example see the loadings plot of the first 2 PC in Figure 3).

Considering a threshold of $\geq$0.20 (absolute value), half of the FA exhibited coefficients exceeding this value in at least 2/3 different PC, whereas 4 FA showed no loading >0.20 for any extracted PCA (Table 1). This was particularly true for PC4, PC5, PC7, and PC9. An interpretation was attempted for the other PC, even if caution should be taken for the interpretation of the PC6 and PC8 due to the low percentage of their explained variance (5.27 and 2.89%, respectively).

The first PC (PC1, variance explained 25.06%) presented the highest loadings for most of the short- and medium-chain FA (negatives), some iso FA, C18:1 *cis*-9, and long-chain SFA (positives). That is in accordance with the effect of carbon chain length on the PC scores, above discussed. Most of these FA are totally or partially synthetized in the mammary gland (Chilliard et al., 2000). Therefore, animals that have large PC1 scores are characterized by a higher content of FA of mammary gland origin; thus, this PC could be considered as an index of FA synthesis in this organ. As an example, Figure 4 reports the averages of some milk FA of animals classified according to PC1 score classes (based on PC1 score distribution quartiles). It can be seen that the average concentration of FA with larger negative loadings on PC1 (e.g., C10:0) decreases across PC1 classes, whereas the FA with positive loadings (e.g., C18:1 *cis*-9) exhibit the opposite trend. Finally, FA with a loading close to zero (e.g., C16:0) do not show a clear pattern. The PC scores could therefore be used as a new individual synthetic phenotype that characterizes animals on the basis of the FA of mammary gland origin. The high positive loadings for PC1 showed by C20:0, C18:1 *cis*-9, and iso C17:0 are also represented in Figure 3, which shows a positive correlation between these FA and PC1. Correlations between C20:0, C18:1 *cis*-9, and iso C17:0 and PC1 were 0.86, 0.80, and 0.75, respectively. On the contrary, C8:0, C10:0, C12:0, and

**Table 1.** Eigenvectors, eigenvalues, and percentage of variance explained of the first 9 principal components (PC) extracted from the correlation matrix of the 49 fatty acids

| | PC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fatty acid[1] | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 |
| C4:0 | −0.054 | 0.143 | 0.006 | −0.205* | 0.123 | 0.041 | −0.287* | −0.160 | 0.099 |
| C6:0 | −0.219* | 0.039 | 0.119 | −0.099 | 0.239* | 0.031 | −0.036 | −0.062 | 0.054 |
| C8:0 | −0.233* | 0.009 | 0.124 | −0.033 | 0.237* | 0.011 | 0.052 | −0.047 | 0.013 |
| C10:0 | −0.239* | −0.044 | 0.124 | 0.015 | 0.216* | −0.005 | 0.110 | −0.006 | 0.036 |
| C10:1 | −0.189 | −0.074 | 0.044 | 0.101 | 0.095 | −0.035 | 0.103 | 0.116 | 0.005 |
| C11:0 | −0.201* | −0.173 | 0.102 | 0.167 | 0.068 | −0.010 | 0.042 | 0.056 | −0.048 |
| C12:0 | −0.228* | −0.094 | 0.109 | 0.068 | 0.190 | −0.022 | 0.151 | 0.045 | 0.019 |
| iso C13:0 | 0.192 | 0.019 | 0.113 | −0.114 | −0.042 | −0.072 | 0.099 | 0.137 | 0.143 |
| anteiso C13:0 | −0.094 | −0.246* | 0.071 | 0.217* | −0.029 | −0.051 | 0.096 | 0.181 | −0.032 |
| iso C14:0 | 0.198 | −0.008 | 0.155 | 0.096 | 0.054 | −0.100 | −0.065 | 0.013 | 0.281* |
| C14:0 | −0.170 | −0.206* | 0.092 | 0.005 | 0.011 | −0.021 | 0.091 | 0.198 | 0.174 |
| iso C15:0 | 0.210* | 0.044 | 0.134 | 0.030 | 0.024 | −0.213* | 0.033 | 0.063 | 0.004 |
| anteiso C15:0 | 0.090 | 0.128 | 0.198 | 0.193 | 0.101 | −0.278* | −0.057 | 0.041 | −0.027 |
| C14:1 *cis*-9 | −0.011 | −0.288* | −0.010 | 0.188 | −0.199 | −0.010 | −0.022 | 0.172 | −0.008 |
| C15:0 | 0.019 | 0.049 | 0.224* | 0.275* | 0.040 | −0.098 | −0.019 | 0.110 | 0.146 |
| iso C16:0 | 0.151 | 0.048 | 0.130 | 0.186 | 0.180 | −0.137 | −0.149 | 0.059 | 0.136 |
| C16:0 | 0.038 | −0.245* | −0.001 | −0.118 | −0.199 | 0.087 | −0.147 | 0.031 | 0.249* |
| iso C17:0 | 0.214* | 0.035 | −0.035 | 0.092 | 0.183 | −0.131 | 0.032 | 0.025 | −0.207* |
| C16:1 *trans*-9 | −0.114 | 0.213 | 0.023 | 0.106 | −0.202* | −0.180 | 0.077 | −0.311* | 0.147 |
| anteiso C17:0 | 0.127 | 0.105 | 0.096 | 0.241* | 0.249* | −0.148 | −0.060 | −0.014 | −0.211* |
| C16:1 *cis*-9 | 0.039 | −0.248* | −0.024 | 0.194 | −0.289* | 0.018 | −0.108 | 0.036 | −0.103 |
| C17:0 | 0.126 | 0.052 | 0.212* | 0.205* | 0.127 | 0.120 | 0.088 | 0.014 | 0.037 |
| C17:1 *cis*-9 | 0.133 | −0.103 | 0.076 | 0.281* | −0.147 | 0.032 | −0.022 | −0.083 | −0.196 |
| C18:0 | 0.155 | 0.191 | −0.021 | −0.212* | 0.160 | −0.078 | 0.107 | 0.109 | −0.158 |
| C18:1 *trans*-4 | 0.096 | 0.030 | −0.246* | −0.041 | 0.107 | −0.015 | 0.245* | 0.202* | 0.147 |
| C18:1 *trans*-5 | 0.054 | 0.027 | −0.263* | 0.031 | 0.119 | 0.007 | 0.274* | 0.117 | 0.185 |
| C18:1 *trans*-6+8 | 0.030 | 0.038 | −0.344* | 0.106 | 0.060 | −0.087 | 0.147 | 0.056 | 0.116 |
| C18:1 *trans*-9 | 0.025 | 0.064 | −0.339* | 0.107 | 0.002 | −0.121 | 0.121 | 0.008 | 0.067 |
| C18:1 *trans*-10 | −0.007 | −0.013 | −0.245* | 0.194 | 0.086 | −0.003 | 0.093 | −0.066 | 0.131 |
| C18:1 *trans*-11 | −0.122 | 0.233* | −0.033 | 0.104 | −0.138 | −0.214* | 0.081 | −0.263* | 0.186 |
| C18:1 *trans*-13+t14 | −0.154 | 0.216* | −0.080 | 0.125 | 0.088 | 0.117 | −0.154 | 0.156 | 0.001 |
| C18:1 *cis*-9 | 0.229* | −0.018 | −0.089 | −0.012 | −0.100 | −0.030 | −0.012 | −0.059 | −0.336* |
| C18:1 *cis*-12 | 0.071 | −0.043 | −0.294* | 0.095 | 0.126 | 0.089 | −0.090 | 0.032 | 0.037 |
| C18:1 *trans*-16+*cis*-14 | −0.090 | 0.284* | −0.073 | 0.056 | 0.064 | 0.117 | −0.160 | 0.210* | −0.128 |
| C18:2 *trans*-9,*trans*-12 | −0.030 | 0.013 | −0.159 | 0.253* | 0.033 | 0.152 | 0.031 | 0.001 | 0.205* |
| C18:2 *cis*-9,*trans*-13 | −0.139 | 0.162 | −0.101 | 0.253* | −0.091 | 0.119 | −0.166 | 0.124 | −0.174 |
| C18:2 *cis*-9,*trans*-12 | −0.087 | 0.192 | −0.139 | 0.190 | −0.012 | 0.143 | −0.197 | 0.176 | −0.121 |
| C18:2n-6 | 0.093 | −0.056 | −0.063 | 0.149 | 0.133 | 0.312* | −0.249* | −0.268* | 0.134 |
| C20:0 | 0.245* | 0.003 | 0.010 | −0.020 | −0.015 | 0.034 | −0.018 | 0.157 | 0.172 |
| C18:3n-6 | 0.020 | −0.205* | −0.001 | 0.076 | 0.193 | 0.118 | −0.103 | −0.150 | 0.125 |
| C18:3n-3 | −0.105 | 0.212* | 0.105 | 0.015 | −0.150 | 0.289* | −0.066 | 0.072 | 0.129 |
| C18:2 *cis*-9,*trans*-11 | −0.111 | 0.150 | −0.027 | 0.193 | −0.267* | −0.224* | 0.076 | −0.306* | 0.085 |
| C22:0 | 0.205* | 0.114 | 0.119 | 0.019 | −0.070 | 0.102 | −0.102 | 0.142 | 0.267* |
| C20:3n-6 | 0.144 | −0.121 | −0.044 | 0.090 | 0.213* | 0.131 | 0.001 | −0.280* | 0.027 |
| C20:4n-6 | 0.153 | −0.160 | −0.019 | 0.064 | 0.193 | 0.141 | 0.059 | −0.326* | −0.077 |
| C20:5n-3 (EPA) | −0.039 | 0.176 | 0.169 | 0.088 | −0.104 | 0.259* | 0.277* | −0.004 | −0.028 |
| C24:0 | 0.189 | 0.147 | 0.127 | −0.002 | −0.066 | 0.118 | −0.070 | 0.092 | 0.205* |
| C22:5n-3 (DPA) | 0.090 | 0.137 | 0.150 | 0.064 | −0.069 | 0.299* | 0.367* | −0.072 | −0.087 |
| C22:6n-3 (DHA) | 0.120 | 0.044 | 0.098 | 0.022 | −0.052 | 0.313* | 0.346* | −0.043 | −0.081 |
| Eigenvalues | 12.28 | 7.38 | 6.55 | 3.84 | 2.61 | 2.58 | 1.53 | 1.42 | 1.26 |
| Variance explained (%) | 25.06 | 15.06 | 13.37 | 7.83 | 5.32 | 5.27 | 3.13 | 2.89 | 2.57 |

[1]EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

*A number with an asterisk indicates the absolute value of eigenvectors ≥0.2 that has been considered the threshold for the association between a original variable to the considered PC.

C6:0 are negatively correlated with PC1 (Table 1 and Figure 3): correlations between these FA and PC1 were −0.82, −0.84, −0.80, and −0.77, respectively.

The PC2 (variance explained 15.06%) had high negative loadings on anteiso C13:0, C14:0, C16:0, C14:1 *cis*-9, C16:1 *cis*-9, and C18:3n-6 and positives on some biohydrogenation (**BH**) products and C18:3n-3 (Table 1 and Figure 3). The association with FA of different origin and metabolic pathways does not allow us to assign a clear meaning to this PC. The only feature shared by FA associated with this PC is their relationship with diet quality, especially with the use of graz-

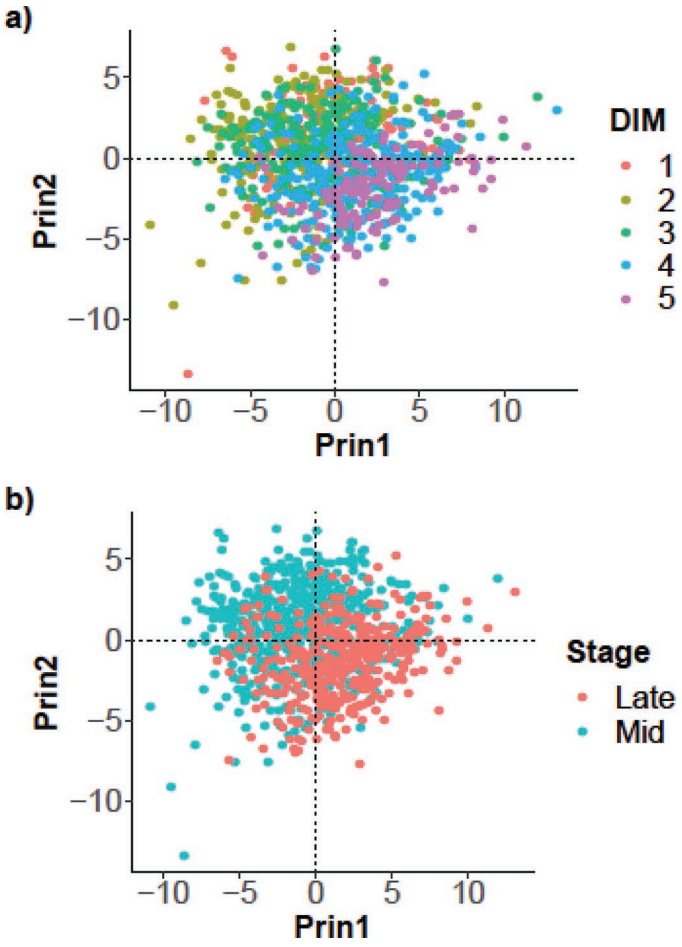


**Figure 1.** Plots of the scores for the first 2 principal components (Prin1 and Prin2; variance explained: 25.06% and 15.06%, respectively) of animals belonging to different classes of DIM (from 1 to 5 in panel a, and averaged in mid and late lactation in panel b).

ing. In dairy cattle (Fievez et al., 2003) the 2 first PC were mostly associated with FA belonging to 4 groups. Two included FA that originate in the mammary gland from de novo synthesis or desaturase activity; the other 2 consist of FA produced in the rumen from BH activity or from microbial synthesis.

The PC3 (variance explained 13.37%) presented high positive loadings for C15:0 and C17:0, and negative for several positional isomers of *trans* C18:1 and on C181 *cis*-12, respectively. This PC could be related to the FA BH processes occurring in the rumen (Shingfield et al., 2010). The PC3 had also high loadings on some odd- and branched-chain FA (**OBCFA**) of microbial origin. The OBCFA profile has been proposed as a useful tool to predict shifts in microbial population associated in particular with the diet (Vlaeminck et al., 2006). The PC6 (variance explained 5.27%) showed the largest positive loadings for PUFA n-3 (DHA, DPA C18:3n-3, EPA) and C18:2n-6, but negative loadings for C18:1 *trans*-11 and C18:2 *cis*-9,*trans*-11 [i.e., the substrates (with positive loadings) and products (with negative loadings) of ruminal FA BH]. This pattern is confirmed by the correlations between these FA and PC; positive correlations were observed for C18:2n-6 (0.51), DHA (0.50), DPA (0.48), C18:3n-3 (0.46), and EPA (0.42), but negative correlations for C18:2 *cis*-9,*trans*-11 (−0.36) and C18:1 *trans*-11 (−0.34). The FA profile of animals that have large PC6 scores is characterized by low content in PUFA and high content in 2 of their BH products. Thus, PC6 could be considered as an indicator of PUFA BH activity in the rumen: the lower the PC6 scores the higher the BH activity. The PC8 (variance explained 2.89%) had large positive loadings on C14:0, C18:1*trans*-4, and 18:1 *trans*-16+*cis*-14, and negative on C16:1 *trans*-9, C18:1*trans*-11, C18:2n-6, C18:2*cis*-9 ,*trans*-11, C20:3n-6, and C20:4n-6 (negatives). Considering the high loadings exhibited by PUFA n-6 and by the main products of the BH of C18:2n-6 (C18:1

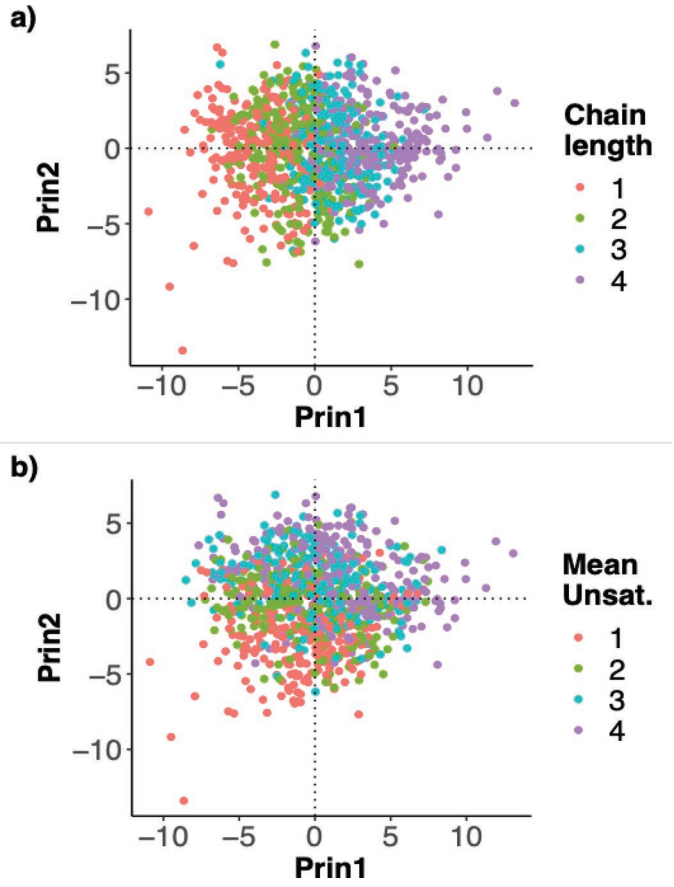


**Figure 2.** Plots of the scores for the first 2 principal components (Prin1 and Prin2; variance explained: 25.06% and 15.06%, respectively) of animals belonging to different classes (from 1 to 4) of mean carbon chain length (a) and of mean unsaturation level (b).

*trans*-11 and C18:2 *cis*-9,*trans*-11), this PCA could be interpreted as an indicator of PUFA n-6 in the diet.

In previous studies on milk FA, PCA was effective in grouping animals according to diet they were fed (Bernard et al., 2009; Correddu et al., 2016). Principal component analysis was also applied on lamb meat FA to differentiate animals according to their geographical origin (Díaz et al., 2005), or to study the relationship between quality traits of carcass and meat of light lambs (Cañeque et al., 2004). Such a different discriminating power among studies could be ascribed to the amount of variance accounted for by the first 2 PC: 40% in the present study and 90% in the paper of Correddu et al. (2016).

### Factor Analysis

The suitability of the data set to the theoretical assumptions of the MFA was assessed through the calculation of the Kaiser measure of sampling adequacy (**MSA**). This index estimates the decrease of partial correlations compared with Pearson correlations between the observed variables. In the present work, the measure of sampling adequacy was 0.75, close to the value of 0.80 indicated as the optimal threshold for the suitability of a data set to MFA (Cerny and Kaiser, 1977). This result was similar to previous reports on the use of MFA on milk FA profile (Mele et al., 2016; Correddu et al., 2017). Nine factors able to explain

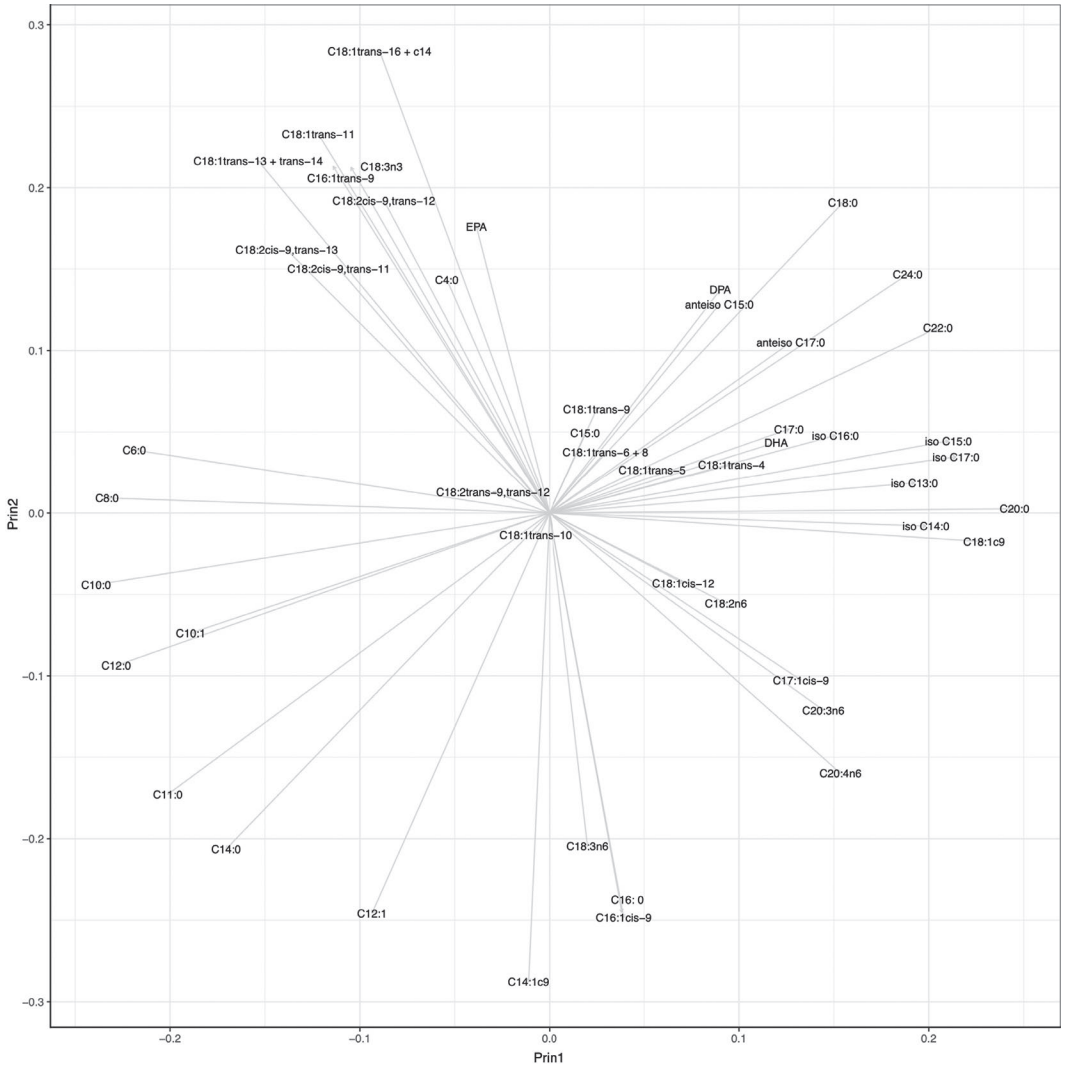


**Figure 3.** Correlation circle. Plot of the loadings of the 2 principal components (Prin1 and Prin2; variance explained: 25.06% and 15.06%, respectively).

about 80% of the total variance of the system were extracted (Table 2). The pattern of explained variance across the different factors was smoother compared with PC (Table 1).

The communality of original variables was on average 0.81 (±0.11), similar to the value reported for buffalo (0.79; Correddu et al., 2017) and higher than in cattle (0.69; Conte et al., 2016; Mele et al., 2016). The 2 FA with the lowest value of communality (0.54 for C18:2 *trans*-9,*trans*-12 and C18:3n-6) were the same as reported in a work on buffalo (C18:2 *trans*-9,*trans*-12 and C18:3n-6; Correddu et al., 2017). Therefore, in both species, these 2 FA are characterized by about 50% of independent variation. Largest communalities, in agreement with previous studies, have been found for short- and medium-chain SFA (e.g., C6:0, C8:0, C10:0, C12:0), associated with the first or second latent factor. The high values observed for these FA, and the agreement among studies, confirm that the variability of these FA is mostly related to a unique metabolic pathway, similar among species.

The adequateness of the factor model for fitting the FA correlation matrix was confirmed by the simple structure of the rotated pattern (Morrison, 1976). In particular, each factor showed large loadings with few variables and small loadings with the other variables (Table 2), respectively. Each variable had a large loading in only one factor, with only one exception (C16:0). In total, 42 out of 49 FA exhibited a loading value ≥0.60, considered as an empirical threshold for declaring a variable associated with a factor (Macciotta et al., 2015). The statistical difference of each factor loading from 0.60 was tested according to Browne et al. (2008).

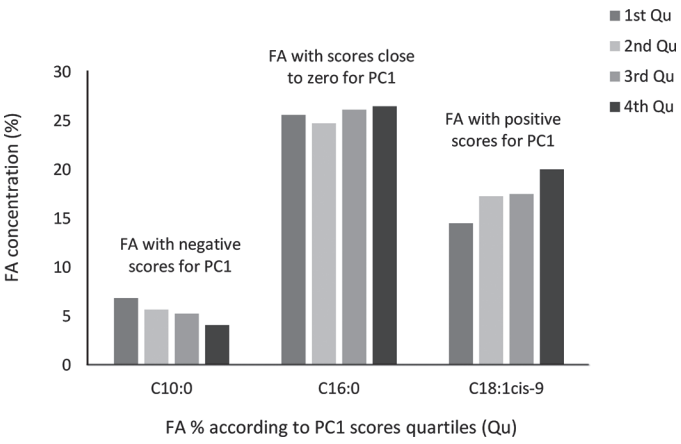


**Figure 4.** Relationship between fatty acid (FA) concentration (%) and principal component (PC) 1 scores for 3 FA: C10:0, C16:0, and C18:1 *cis*-9, chosen as representative FA with negative, close to zero, and positive loadings for PC1, respectively.

The first latent factor (**F1**) was positively correlated with short- and medium-chain FA (apart from C4:0 and C16:0) and negatively with C18:1 *cis*-9 and some long-chain SFA (C20:0, C22:0, and C24:0). Thus, it was considered an index of "mammary gland FA synthesis." A peculiarity of F1 is its structural similarity with PC1. A concordance between the results of the first PC and the first factor extracted from the same data set was observed in a study on body conformation traits in cows (Olasege et al., 2019). The F1 structure partially agrees with previous studies where it was associated with mammary gland ability to maintain an optimal milk fat fluidity and with the FA neosynthesis (Conte et al., 2016; Correddu et al., 2017; Palombo et al., 2020). The negative loadings of F1 for long-chain SFA (C20:0, C22:0, and C24:0) was not observed in previous studies. In a recent investigation on Comisana sheep, they were associated with a factor interpreted as branched fatty acid metabolism (Palombo et al., 2020). In cows they were associated with a different factor together with other saturated and unsaturated long-chain FA (Conte et al., 2016; Mele et al., 2016), whereas in buffalo they characterized a specific factor (Correddu et al., 2017). These results could be partially explained by the sampling effect. However, some differences among species could exist, especially related to the farming system.

Being positively associated with the odd, iso, and anteiso FA (except iso C13:0), F2 was named OBCFA. These FA are almost completely synthesized by rumen microorganisms (Vlaeminck et al., 2006). This result is in agreement with a previous report on sheep (Palombo et al., 2020), whereas 2 distinct factors associated with odd-chain FA (**OCFA**) and branched-chain FA (**BCFA**) were found in cattle and buffalo (Conte et al., 2016; Correddu et al., 2017). The relative milk concentration of these FA depends on the composition of the microbial population (Vlaeminck et al., 2006). The diet, especially its forage to concentrate ratio, is one of the main factors affecting the relative abundance of microbial populations. Thus, feeding management could affect the proportions of OCFA and BCFA in milk. Sheep involved in the present study are farmed in the typical Mediterranean semi-extensive systems with pasture as main feeding source (Macciotta et al., 1999; Molle et al., 2007). Under these conditions, forage to concentrate ratio in the diet should be approximately similar in the various flocks and, therefore, also the rumen microbial composition to a certain extent. As a consequence, the correlation pattern of all OBCFA is similar, and the underling pathway of variation is summarized in one unique latent factor.

Factor 3 and 4 were positively associated with all isomers of C18:1 and C18:2 originating from the ruminal BH of PUFA, with the exception of C18:1 *trans*-11

**Table 2.** Rotated factor pattern and communality

| Fatty acid[1] | Factor[2] | | | | | | | | | Com[3] |
|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | |
| C12:0 | 0.95* | −0.06 | −0.11 | 0.03 | 0.06 | 0.02 | −0.01 | −0.06 | −0.03 | 0.94 |
| C10:0 | 0.95* | −0.08 | −0.19 | 0.06 | −0.11 | 0.06 | 0.00 | −0.06 | −0.01 | 0.96 |
| C8:0 | 0.87* | −0.09 | −0.24 | 0.12 | −0.28 | 0.07 | −0.01 | −0.05 | −0.03 | 0.93 |
| C11:0 | 0.83* | −0.05 | −0.17 | 0.06 | 0.41 | 0.01 | −0.08 | 0.03 | −0.03 | 0.91 |
| C6:0 | 0.77* | −0.13 | −0.29 | 0.14 | −0.42 | 0.05 | −0.05 | −0.03 | 0.05 | 0.89 |
| C10:1 | 0.73* | −0.06 | 0.00 | 0.12 | 0.17 | 0.04 | −0.05 | −0.12 | −0.01 | 0.59 |
| C14:0 | 0.73* | −0.17 | −0.12 | −0.17 | 0.35 | −0.13 | −0.11 | −0.14 | 0.25 | 0.83 |
| iso C13:0 | −0.48 | 0.36 | −0.08 | −0.41 | −0.08 | −0.20 | 0.18 | −0.18 | 0.17 | 0.68 |
| C24:0 | −0.58 | 0.45 | −0.15 | 0.01 | −0.18 | −0.08 | 0.35 | 0.01 | 0.32 | 0.82 |
| C22:0 | −0.60* | 0.49 | −0.11 | −0.02 | −0.10 | −0.13 | 0.29 | 0.03 | 0.40 | 0.88 |
| C20:0 | −0.66* | 0.37 | 0.14 | −0.25 | 0.02 | −0.31 | 0.13 | 0.07 | 0.21 | 0.82 |
| C18:1 *cis*-9 | −0.79* | 0.10 | 0.11 | −0.18 | 0.16 | −0.18 | 0.02 | 0.10 | −0.37 | 0.88 |
| anteiso C15:0 | −0.08 | 0.86* | −0.19 | 0.01 | −0.06 | 0.20 | 0.01 | −0.14 | −0.13 | 0.85 |
| iso C16:0 | −0.20 | 0.81* | −0.03 | −0.05 | −0.02 | −0.06 | −0.04 | 0.16 | 0.06 | 0.73 |
| anteiso C17:0 | −0.15 | 0.80* | 0.02 | 0.12 | −0.07 | −0.01 | 0.04 | 0.14 | −0.38 | 0.84 |
| C15:0 | 0.19 | 0.72* | −0.20 | 0.10 | 0.19 | 0.16 | 0.16 | −0.03 | 0.14 | 0.72 |
| iso C14:0 | −0.35 | 0.69* | −0.08 | −0.33 | 0.06 | −0.07 | 0.07 | 0.15 | 0.24 | 0.82 |
| C17:0 | −0.07 | 0.67* | −0.16 | −0.02 | 0.05 | −0.10 | 0.48 | 0.22 | 0.03 | 0.76 |
| iso C15:0 | −0.47 | 0.66* | −0.08 | −0.34 | −0.02 | −0.07 | 0.07 | −0.12 | −0.07 | 0.81 |
| iso C17:0 | −0.48 | 0.53 | 0.26 | −0.14 | −0.05 | −0.22 | 0.00 | 0.11 | −0.37 | 0.80 |
| C18:1 *trans*-6 + 8 | −0.18 | −0.12 | 0.89* | 0.14 | 0.00 | 0.10 | −0.19 | 0.02 | −0.08 | 0.92 |
| C18:1 *trans*-9 | −0.23 | −0.14 | 0.83* | 0.17 | 0.00 | 0.21 | −0.21 | −0.02 | −0.13 | 0.90 |
| C18:1 *trans*-5 | −0.13 | −0.08 | 0.82* | −0.02 | −0.10 | −0.08 | 0.03 | 0.00 | 0.02 | 0.71 |
| C18:1 *trans*-4 | −0.27 | −0.05 | 0.76* | −0.08 | −0.14 | −0.21 | 0.01 | −0.10 | 0.02 | 0.73 |
| C18:1 *trans*-10 | 0.04 | −0.06 | 0.68* | 0.15 | 0.13 | 0.15 | −0.11 | 0.25 | −0.05 | 0.60 |
| C18:1 *cis*-12 | −0.25 | −0.12 | 0.65* | 0.18 | 0.07 | −0.20 | −0.22 | 0.35 | −0.05 | 0.75 |
| C18:2 *trans*-9,*trans*-12 | 0.11 | 0.00 | 0.49 | 0.34 | 0.19 | 0.13 | 0.08 | 0.29 | 0.15 | 0.54 |
| C18:2 *cis*-9,*trans*-13 | 0.16 | −0.08 | 0.11 | 0.87* | 0.11 | 0.27 | 0.03 | −0.06 | −0.07 | 0.90 |
| C18:2 *cis*-9,*trans*-12 | 0.01 | −0.04 | 0.22 | 0.86* | −0.07 | 0.13 | 0.00 | −0.03 | −0.02 | 0.81 |
| C18:1 *trans*-16 + *cis*-14 | 0.02 | 0.02 | 0.09 | 0.82* | −0.41 | 0.08 | 0.08 | −0.21 | −0.03 | 0.91 |
| C18:1 *trans*-13 + *trans*-14 | 0.29 | −0.03 | 0.14 | 0.80* | −0.29 | 0.17 | 0.01 | −0.09 | 0.07 | 0.86 |
| C18:3n-3 | 0.09 | −0.11 | −0.30 | 0.56 | −0.23 | 0.21 | 0.43 | −0.12 | 0.36 | 0.85 |
| C14:1 *cis*-9 | 0.14 | −0.08 | 0.02 | −0.14 | 0.88* | −0.16 | −0.16 | 0.07 | 0.10 | 0.89 |
| C16:1 *cis*-9 | −0.14 | −0.10 | −0.07 | −0.09 | 0.88* | −0.05 | −0.14 | 0.17 | 0.01 | 0.87 |
| C12:1 *cis*-9 | 0.55 | 0.06 | −0.02 | −0.10 | 0.71* | −0.12 | −0.08 | 0.00 | 0.00 | 0.84 |
| C17:1 *cis*-9 | −0.30 | 0.35 | −0.11 | −0.04 | 0.62* | 0.02 | 0.18 | 0.28 | −0.19 | 0.75 |
| C18:0 | −0.50 | 0.22 | 0.13 | −0.10 | −0.61* | −0.23 | 0.13 | −0.27 | −0.23 | 0.89 |
| C4:0 | 0.00 | −0.14 | −0.23 | 0.17 | −0.63* | 0.07 | −0.19 | 0.08 | 0.13 | 0.57 |
| C18:2 *cis*-9,*trans*-11 | 0.08 | 0.00 | 0.04 | 0.22 | 0.09 | 0.92* | −0.02 | −0.17 | −0.05 | 0.93 |
| C16:1 *trans*-9 | 0.10 | 0.02 | −0.05 | 0.21 | −0.17 | 0.88* | 0.07 | −0.19 | 0.03 | 0.91 |
| C18:1 *trans*-11 | 0.13 | 0.03 | 0.11 | 0.25 | −0.26 | 0.86* | −0.01 | −0.22 | 0.03 | 0.95 |
| C22:5n-3 (DPA) | −0.20 | 0.17 | −0.12 | 0.03 | −0.08 | 0.04 | 0.88* | 0.03 | −0.05 | 0.87 |
| C22:6n-3 (DHA) | −0.25 | 0.07 | −0.04 | −0.11 | 0.02 | −0.15 | 0.77* | 0.12 | −0.03 | 0.71 |
| C20:5n-3 (EPA) | 0.11 | 0.09 | −0.23 | 0.27 | −0.10 | 0.20 | 0.75* | −0.12 | 0.07 | 0.78 |
| C18:2n-6 | −0.20 | 0.06 | 0.10 | 0.14 | 0.06 | −0.13 | 0.06 | 0.80* | 0.13 | 0.76 |
| C20:4n-6 | −0.18 | 0.12 | 0.12 | −0.39 | 0.13 | −0.25 | 0.13 | 0.67* | −0.24 | 0.81 |
| C20:3n-6 | −0.18 | 0.17 | 0.20 | −0.28 | 0.07 | −0.21 | 0.07 | 0.66* | −0.13 | 0.68 |
| C18:3n-6 | 0.21 | 0.03 | 0.04 | −0.22 | 0.20 | −0.25 | −0.12 | 0.56 | 0.07 | 0.54 |
| C16:0 | −0.05 | −0.07 | −0.04 | −0.04 | 0.06 | 0.00 | −0.07 | 0.04 | 0.42* | 0.75 |
| Eigenvalue | 8.92 | 5.47 | 4.79 | 4.74 | 4.70 | 3.47 | 3.04 | 2.81 | 1.53 | |
| Variance explained (%) | 17.62 | 10.80 | 9.46 | 9.36 | 9.29 | 6.86 | 6.00 | 5.54 | 3.01 | |

[1]EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid.

[2]F1 = mammary gland FA synthesis; F2 = odd- and branched-chain fatty acids; F3 = biohydrogenation; F4 = LNA (α-linolenic acid) BH; F5 = desaturase; F6 = CLA; F7 = n-3; F8 = n-6; F9 = C16.

[3]Communality.

*Absolute value of factor loadings ≥0.60 that were considered significant for the interpretation of the factor pattern.

(vaccenic acid) and C18:2 *cis*-9,*trans*-11 (rumenic acid). In particular, F3 was associated with *trans* isomer of C18:1 from the fourth to the tenth position, C18:1*cis* -12, and to a lesser extent, with C18:2 *trans*-9,*trans*-12. Factor 4 was associated with *trans* isomer of C18:1 from the 13th to the 16th position, C18:2 *cis*-9,*trans*-12, C18:2 *cis*-9,*trans*-13, and C18:3 *cis*-9,*cis*-12,*cis*-15 (C18: 3n-3, α-linolenic acid, **LNA**). Although it is very dif-

ficult to unequivocally ascertain the metabolic origin of a specific minor BH intermediate (Shingfield et al., 2010), the separation of these FA into 2 different latent factors can suggest different metabolic pathways underlying the BH of PUFA. In particular, FA associated with the 3th factor are often produced in the rumen during the BH process of C18:2 *cis*-9,*cis*-12 (C18:2n -6, linoleic acid; Shingfield et al., 2010). This result is in agreement with a previous report in cattle where an association of C18:2n-6 and its intermediate products in the same latent factor was found (Mele et al., 2016). In the present study C18:2 *cis*-9,*cis*-12 was not associated with F3 and, consequently, we decided to assign the generic name of BH. Considering the association of C18:3n-3 and of some of its ruminal BH intermediates with the F4, this factor was named LNA-BH. Almost all FA here found to be associated with F3 and F4 were found in a single latent factor, together with vaccenic and rumenic acids, in previous studies on cattle, buffalo, and sheep (Conte et al., 2016; Correddu et al., 2017; Palombo et al., 2020).

The fifth latent factor was named desaturase, being positively associated with some products of stearoyl CoA desaturase (**SCD**) activity (C12:1 *cis*-9, C14:1 *cis*-9, C16:1 *cis*-9, and C17:1 *cis*-9) and negatively with the preferred substrate of this enzyme (C18:0). The other SCD products, C18:1 *cis*-9 and C18:2 *cis*-9,*trans*-11, were highly correlated with the first and seventh latent factors, respectively. This result is in agreement with previous investigations in buffalo (Correddu et al., 2017) and, partially, in cattle (Conte et al., 2016; Mele et al., 2016), where the C17:1 *cis*-9 was not associated with the factor related to SCD activity, but with the same factor including C18:1 *cis*-9. Results of the present study are also in partial agreement with a previous report in sheep (Palombo et al., 2020). However, in this study the C17:1 *cis*-9 did not correlate with any factor. Interestingly, the desaturase factor presented a high loading value for C4:0 (−0.63), which is different from previous studies where this FA was associated with a factor of C6:0 (Mele et al., 2016), or was not associated with any factor (Conte et al., 2016; Correddu et al., 2017).

Factor 6 was named CLA because it showed large correlations with C18:2 *cis*-9,*trans*-11 (rumenic acid) and C18:1 *trans*-11 (vaccenic acid). It was associated with synthesis of the most abundant and important milk CLA isomer (C18:2 *cis*-9,*trans*-11) operated by the SCD in the mammary gland. Rumenic and vaccenic acids are of great importance in the nutritional quality of milk (Banni et al., 2003) and much research has been aimed at finding strategies for increasing their concentration (Chilliard et al., 2001; Nudda et al., 2014). The milk of animals with higher CLA factor scores is richer in these FA, with an improvement of its nutritional value. The partition of the SCD products into 3 different factors is in agreement with the work of Mele et al. (2016), which explained this result with the chain length and the unsaturation degree of the substrate on SCD activity. Conversely, rumenic and vaccenic acids were associated with the BH factor in Comisana sheep (Palombo et al., 2020). In the present study also C16:1 *trans*-9 was correlated with the CLA factor. A similar result, even though to a lesser extent, was reported by Mele et al. (2016). In another work, it was correlated with the factor associated with the long-chain FA (Conte et al., 2016).

The seventh and eighth latent factors were named n-3 and n-6 as they were positively correlated with FA of the PUFA n-3 family and the PUFA n-6 family, respectively. The extraction of 2 different factors for PUFA n-3 and PUFA n-6 is in agreement with a recent report on buffalo (Correddu et al., 2017), whereas in cattle they were associated with a unique latent factor (Conte et al., 2016; Mele et al., 2016). This result could arise from differences in the metabolism of these FA, in particular from the capacity to promote C18:3n-3 and C18:2n-6 elongation, or differences in the dietary concentration of these 2 FA (Correddu et al., 2016). Although their milk concentration is not high (0.5% of total FA, n-3 + n-6 excluding C18:3n-3 and C18:2n-6), these FA have great nutritional importance (Connor, 2000). In particular high concentrations of PUFA along with a low n-6 to n-3 ratio are considered important for good health and normal development in humans (Simopoulos, 2002). The ninth factor explained 3% of the total variance and did not show relevant loading values.

### Mixed-Model Analysis

Results of the mixed-model analysis carried out on the individual scores of the 9 PC and of the 9 extracted factors are reported in Table 3.

### Principal Components

On average, the contribution of the flock to the PC variance was around 46%, with the highest value exhibited by PC3 (69%) and the lowest by PC8 (31%). The high contribution of the flock to the variance of PC3 could arise from the great influence of environmental factors such as diet, climate, and farming practices on the ruminal microbial environment (Henderson et al., 2015), which, in turn, influences the FA BH process and the production of OBCFA. For similar reasons, a low contribution of flock to the PC8 variance was not expected, with this PC being interpreted as an indicator of PUFA n-6 in the diet.

**Table 3.** Effect of DIM, parity, month, and number of lambs born, and altitude of flock on the 9 principal components and 9 latent factors

| Item | *P*-value | | | | | |
|---|---|---|---|---|---|---|
| | DIM | Parity | Lambing month | Lambs born | Altitude | $r^2_{f(ALT)}$ [1] |
| Principal component (PC) | | | | | | |
| PC1 | <0.001* | <0.001* | <0.001* | 0.683 | 0.469 | 0.53 |
| PC2 | <0.001* | 0.647 | 0.413 | 0.213 | 0.831 | 0.53 |
| PC3 | 0.762 | 0.635 | 0.249 | 0.267 | 0.545 | 0.69 |
| PC4 | 0.067 | 0.157 | 0.072 | 0.934 | 0.407 | 0.36 |
| PC5 | 0.195 | 0.008* | 0.006* | 0.177 | 0.343 | 0.42 |
| PC6 | 0.153 | 0.006* | 0.029* | 0.744 | 0.526 | 0.51 |
| PC7 | 0.187 | 0.180 | 0.469 | 0.079 | 0.156 | 0.39 |
| PC8 | 0.186 | 0.018* | 0.691 | 0.209 | 0.938 | 0.31 |
| PC9 | 0.032* | 0.688 | <0.001* | 0.337 | 0.042* | 0.37 |
| Latent factor (F)[2] | | | | | | |
| F1: Mammary FA synthesis | <0.001* | 0.022* | <0.001* | 0.860 | 0.921 | 0.43 |
| F2: OBCFA | <0.001* | <0.001* | <0.001* | 0.559 | 0.907 | 0.49 |
| F3: Biohydrogenation | 0.137 | 0.800 | 0.025* | 0.486 | 0.596 | 0.53 |
| F4: LNA-BH | <0.001* | 0.588 | <0.001* | 0.059 | 0.222 | 0.39 |
| F5: Desaturase | <0.001* | 0.614 | 0.143 | 0.187 | 0.425 | 0.25 |
| F6: CLA | <0.001* | 0.209 | 0.002* | 0.350 | 0.583 | 0.40 |
| F7: n-3 | 0.062 | 0.001* | 0.213 | 0.140 | 0.445 | 0.55 |
| F8: n-6 | 0.122 | 0.007* | <0.001* | 0.901 | 0.501 | 0.50 |
| F9: C16 | 0.004* | 0.500 | 0.016* | 0.175 | 0.031* | 0.52 |

[1] $r^2_{f(ALT)}$ = proportion of variance explained by the random effect of flock.

[2] FA, fatty acid; BCFA = odd- and branched-chain fatty acids; LNA-BH = α-linolenic acid (C18:3 *cis*-9,*cis*-12,*cis*-15) biohydrogenation; n-3 = PUFA belonging to the n-3 family; n-6 = PUFA belonging to the n-6 family; C16 = palmitic acid (C16:0).

*$P < 0.05$.

The DIM class significantly affected PC1, PC2, and PC9 (Table 3). Least squares means of PC1 scores exhibited an increasing trend across lactation stages (Figure 5). This trend underlines a reduction in de novo FA synthesis as the lactation proceeds (they have negative loadings) together with an increase of C18:1*cis*-9 synthesis, in agreement with the reports of Timmen and Patton (1988). Although the same trend was observed for PC9, its scores of this PC were lower compared with PC1. Whereas the PC2 showed an opposite pattern (Figure 5).

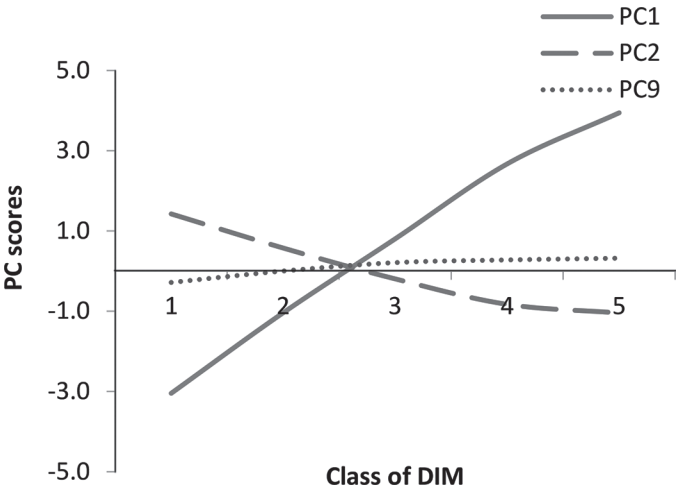


**Figure 5.** Least squares means of principal component (PC) 1, PC2, and PC9 scores for classes of DIM.

Parity significantly affected PC1, PC5, PC6, and PC8. First lambing ewes exhibited the largest least squares mean of PC1 scores (Table 4), which was statistically different from later parities. The PC5 scores decreased across parities, even if with some fluctuations. Scores of PC6 decreased from the first to the fifth parity and then increased until the seventh, whereas PC8 showed the opposite behavior (Table 4). Interestingly, the effect of parity on PC6 underlines a high concentration of both n-3 and n-6 PUFA in primiparous sheep, followed by a decrease in the intermediate parities and then by an increase in the last parities. Similarly to other milk composition traits, FA profile is affected by parity due to changes in energy and overall metabolism of the ewes as the lactation number proceeds (González-García et al., 2015). The results of the present study partially agree with previous research that found higher proportions of more desirable FA in milk of first parity compared with later parities both in sheep and cows (Mierlita et al., 2011; Bilal et al., 2014). The larger content of favorable FA especially in first-parity animals is also in agreement with the pattern of PC8 scores (Table 4).

The lambing month significantly affected PC1, PC5, PC6, and PC9. Scores for all these PC, except from PC6 (Figure 6), were negative from October to De-

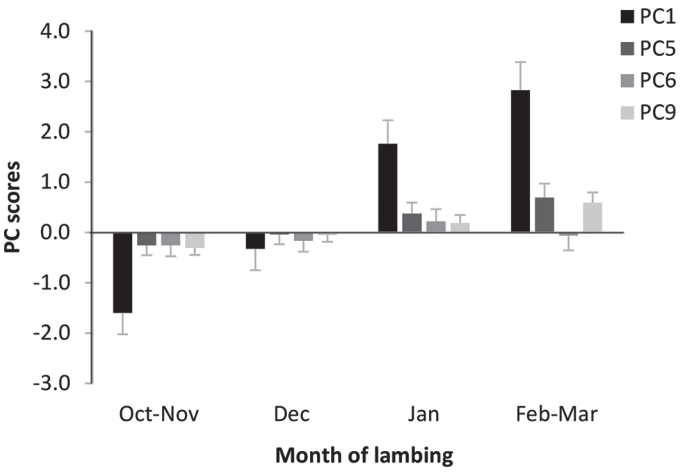


**Figure 6.** Least squares means of principal component (PC) 1, PC5, PC6, and PC9 scores for different lambing months. Errors bars indicate SEM.

cember and positive from January to March. The PC1 exhibited larger absolute values in comparison to PC5 and PC9. Altitude of location of flock affected only PC9 scores, with a decreasing trend passing from plain to mountain. The number of lambs born did not affect any of the 9 PC.

### Latent Factors

Results of the mixed-model analysis factor scores are reported in Table 3. On average, the contribution of the flock effect to the total variance was 45%, with the highest values for the n-3 (55%) and the lowest for the desaturase (25%) factors, respectively. This finding is consistent with the larger effect of environmental and management factors on the milk content of FA arising from the diet (i.e., PUFA) compared with those of endogen production (i.e., MUFA produced by delta-9 desaturase; Stoop et al., 2008; Correddu et al., 2019). According to the high value observed for PC3, the OBCFA and BH factors exhibited high values of variance explained by the flock effect (0.49 and 0.53, respectively).

The number of lambs born and the altitude of flock location did not affect any of the extracted factors. The DIM significantly affected mammary activity, OBCFA, LNA-BH, desaturase, and CLA factor scores. In particular least squares means for scores of mammary activity, LNA-BH, and CLA decreased along the lactation, whereas OBCFA and desaturase exhibited an opposite trend (Figure 7). The effect of DIM class on the mammary activity factor confirmed results obtained for PC1. The higher contents of de novo FA and lower of C18:1 *cis*-9 in early compared with late lactation evidenced by F1 pattern (Figure 7) are in agreement with previous reports in buffalo (Correddu et al., 2017). In dairy cows, a different behavior was observed (Conte et al., 2016; Mele et al., 2016). Such differences could be partially ascribed to differences in the metabolism among species, even if the data distribution along the lactation should also be considered. In the typical Mediterranean sheep farming system, the milk of the first month of lactation is suckled by the lamb. Thus, milk tests considered in the present work were available only from 45 d after parturition. The lack of data for the first month could have therefore hampered the modeling of a trend of FA metabolic pathway in early lactation. Lactation patterns of LNA-BH and CLA factors evidenced a trend similar to mammary gland FA synthesis. Such a decreasing pattern underlined a higher activity of LNA ruminal BH and of CLA synthesis (due to the increase of SCD substrate C18:1 *trans*-11) in the first part of lactation compared with the last part. This finding was in agreement to that observed for the PC2, and it could be explained by the high content of C18:3n-3 in spring Mediterranean pastures (Cabiddu et al., 2005), which tends to decrease as in late spring-summer. The pattern of the desaturase factor underlines an increasing SCD

**Table 4.** Least squares means (±SE) of the principal components affected by parity

| | Principal component (PC) | | | |
|---|---|---|---|---|
| Parity | PC1 | PC5[1] | PC6 | PC8 |
| 1 | $1.98^{a} \pm 0.45$ | $0.54 \pm 0.21$ | $0.29^{a} \pm 0.23$ | $-0.09^{ab} \pm 0.15$ |
| 2 | $0.60^{b} \pm 0.45$ | $0.30 \pm 0.21$ | $0.03^{ab} \pm 0.23$ | $0.10^{ab} \pm 0.16$ |
| 3 | $0.30^{b} \pm 0.44$ | $0.44 \pm 0.21$ | $-0.26^{ab} \pm 0.23$ | $0.08^{ab} \pm 0.15$ |
| 4 | $0.53^{b} \pm 0.44$ | $0.34 \pm 0.20$ | $-0.27^{b} \pm 0.23$ | $0.27^{a} \pm 0.15$ |
| 5 | $0.47^{b} \pm 0.45$ | $0.28 \pm 0.21$ | $-0.28^{ab} \pm 0.23$ | $0.07^{ab} \pm 0.16$ |
| 6 | $0.42^{b} \pm 0.46$ | $0.02 \pm 0.22$ | $-0.03^{ab} \pm 0.24$ | $-0.04^{ab} \pm 0.16$ |
| 7 | $0.56^{b} \pm 0.49$ | $-0.03 \pm 0.24$ | $0.16^{ab} \pm 0.26$ | $-0.20^{b} \pm 0.18$ |
| 8 | $0.49^{ab} \pm 0.64$ | $-0.35 \pm 0.32$ | $-0.17^{ab} \pm 0.34$ | $-0.32^{ab} \pm 0.26$ |

[a,b]Least squares means with different superscript letters within a column differ ($P < 0.05$).

[1]Although PC5 was significantly affected by parity, differences among contrasts did not reach statistical significance ($\alpha = 0.05$).

**Table 5.** Least squares means (±SE) of the latent factors affected by parity

| | Latent factor[1] | | | |
|---|---|---|---|---|
| Parity | Mammary FA synthesis | OBCFA | n-3 | n-6 |
| 1 | $-0.37^{b} \pm 0.13$ | $0.23^{ab} \pm 0.14$ | $0.09^{ab} \pm 0.14$ | $0.35^{a} \pm 0.14$ |
| 2 | $-0.06^{ab} \pm 0.13$ | $0.15^{ab} \pm 0.15$ | $-0.03^{abc} \pm 0.14$ | $0.11^{ab} \pm 0.14$ |
| 3 | $0.04^{a} \pm 0.13$ | $0.23^{a} \pm 0.14$ | $-0.24^{c} \pm 0.14$ | $0.08^{ab} \pm 0.13$ |
| 4 | $-0.04^{ab} \pm 0.13$ | $0.21^{a} \pm 0.14$ | $-0.21^{bc} \pm 0.14$ | $-0.07^{b} \pm 0.13$ |
| 5 | $-0.08^{ab} \pm 0.13$ | $0.08^{abc} \pm 0.15$ | $-0.15^{abc} \pm 0.14$ | $-0.05^{b} \pm 0.14$ |
| 6 | $-0.10^{ab} \pm 0.14$ | $-0.01^{abc} \pm 0.15$ | $0.05^{a} \pm 0.15$ | $0.01^{ab} \pm 0.14$ |
| 7 | $-0.16^{ab} \pm 0.15$ | $-0.15^{bc} \pm 0.16$ | $0.06^{abc} \pm 0.16$ | $0.15^{ab} \pm 0.15$ |
| 8 | $-0.29^{ab} \pm 0.20$ | $-0.45^{c} \pm 0.21$ | $-0.14^{abc} \pm 0.20$ | $-0.01^{ab} \pm 0.20$ |

[a–c]Least squares means with different superscript letters within a column differ ($P < 0.05$).

[1]OBCFA = odd- and branched-chain fatty acids; n-3 = PUFA belonging to the n-3 family; n-6 = PUFA belonging to the n-6 family.

activity as the lactation proceeds, as observed in cattle and buffalo (Mele et al., 2016; Correddu et al., 2017). According to Mele et al. (2016), the increasing trend of OBCFA factor along the lactation can be related to the variation of forage to concentrate ratio. A higher amount of concentrate is usually provided in early lactation to meet energy needs of the animals; as the lactation proceeds, there is an increase of the proportion of forages in the diet resulting in an increase of FA produced by the ruminal microorganism, in particular by cellulolytic bacteria (Vlaeminck et al., 2006). Higher scores for BCFA factors were observed in cows fed a diet with a higher percentage of forage (Conte et al., 2016).

Parity had a significant effect on mammary activity, OBCFA, n-3, and n-6. Mammary activity exhibited an increasing trend from first to third parity (Table 5) and then decreased until the 8 parity. The OBCFA scores were rather constant from the first to the fourth parity and then rapidly decrease in the seventh and eighth parities. The n-3 and n-6 factors showed a similar waving pattern (Table 5). There is a lack of consensus on the effect parity on latent factors extracted from milk FA. Some works evidenced a large effect (Mele et al., 2016), whereas others showed a minor or no effect (Conte et al., 2016; Correddu et al., 2017). The effect of parity on milk FA is mainly due to the larger PUFA content in primiparous compared with pluriparous animals, which exhibit a higher amount of SFA. These figures have been observed both in cows and sheep (Mierlita et al., 2011; Bilal et al., 2014). Differences between parities in the extent of tissue mobilization and in the content of FA synthase in the mammary gland, as well as the rumen microflora, can partially explain the effect of parity on milk FA (Miller et al., 2006; Friggens et al., 2007). In the present work, first lambing animals exhibited lower scores for mammary activity, and higher for n-3 and n-6 factors, respectively. Scores of the OBCFA factor underlined a decreasing pattern of ruminal derived FA with age, as previously reported in cows and buffalo (Mele et al., 2016; Correddu et al., 2017).

The month of lambing significantly influenced ($P < 0.05$) all the latent factors, except for desaturase and n-3. Mammary activity, LNA-BH, and CLA factors exhibited positive scores for lambings occurring from October to December and negative scores for those from January to March, respectively (Figure 8). An opposite trend could be observed for OBCFA, BH, and n-6. Sheep lambing is strictly seasonal; thus, the evaluation of the effect of lambing month on a productive response has a different meaning in comparison, for example, with dairy cattle.

In the typical farming system of Sarda sheep there is a confounding between lambing season, production

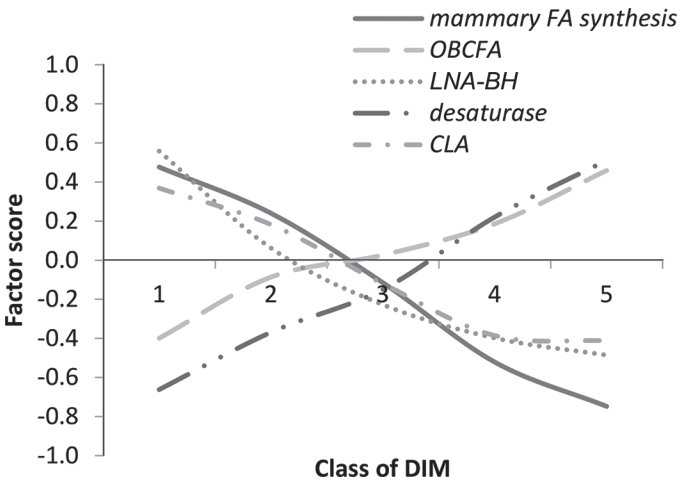


**Figure 7.** Least squares means of mammary fatty acid (FA) synthesis, odd- and branched-chain fatty acid (OBCFA), linolenic acid biohydrogenation (LNA-BH), desaturase, and CLA factor scores for classes of DIM.

season, and parity. Pluriparous ewes lamb in late fall-early winter, whereas first-parity animals lamb in late winter-early spring. Regardless their parity order, the ewes are generally dried off at the beginning of summer. As a consequence, the number of autumn lambing ewes is larger, and they also have longer lactations. Autumn-lambing sheep were sampled in late lactation, whereas winter-lambing sheep were sampled in mid lactation. Thus, the effects on FA profile of the physiological condition of the animal (stage of lactation, parity) and of the environment (mainly pasture quality) on the FA profile are difficult to disentangle. For example, the larger scores for mammary activity found in autumn lambing sheep reflect higher activity of the mammary gland in the FA synthesis in late lactation, whereas winter lambing sheep showed higher content of FA derived from body reserve mobilization in early lactation to meet energy requirements. The lower scores of LNA-BH and CLA factors observed in milk of sheep lambing in winter underline a lower activity of rumen LNA BH, which results in low milk contents of LNA and its BH intermediates, C18:1 *trans*-11 and C18:2 *cis*-9,*trans*-11. This pattern reflects, probably, the lower quality of pastures in late spring compared with late winter-early spring. This finding has interesting implications on the quality of milk in relationship to the season of lambing and to the availability of high-quality pasture, evidencing higher content of desired FA in milk of sheep lambed in autumn.

### Comparison of the 2 Techniques

The comparison of the 2 different dimension-reduction methods for analyzing the FA profile of sheep milk provided interesting insights to assess the usefulness of these 2 multivariate techniques in deciphering complex correlation patterns and in generating new phenotypes that could be further used for management or genetic purposes.

The continuous development of analytical technology has remarkably increased the number of potentially detectable FA. Thus, the number of original variables investigated in the present research was larger in comparison with studies carried out some years ago. In many cases, the newly measured FA were probably not distinguishable from other FA in the previous analyses. Instead of being a simple addition of new variables, this increase of system dimensionality may have added further complexity to the correlation structure of FA. Both PCA and MFA were able to summarize the 49 dimensions of the original multivariate system with 9 new axes that accounted for about 80% of the original variance. Some authors suggest that, when the number of original variables is large, PC and factors tend to coincide (Schneeweiss and Mathes, 1995). However, in the present study, some differences have been found in the meaning of the extracted variables.

In general, PCA structures were difficult to interpret, also in comparison with previous research on milk FA

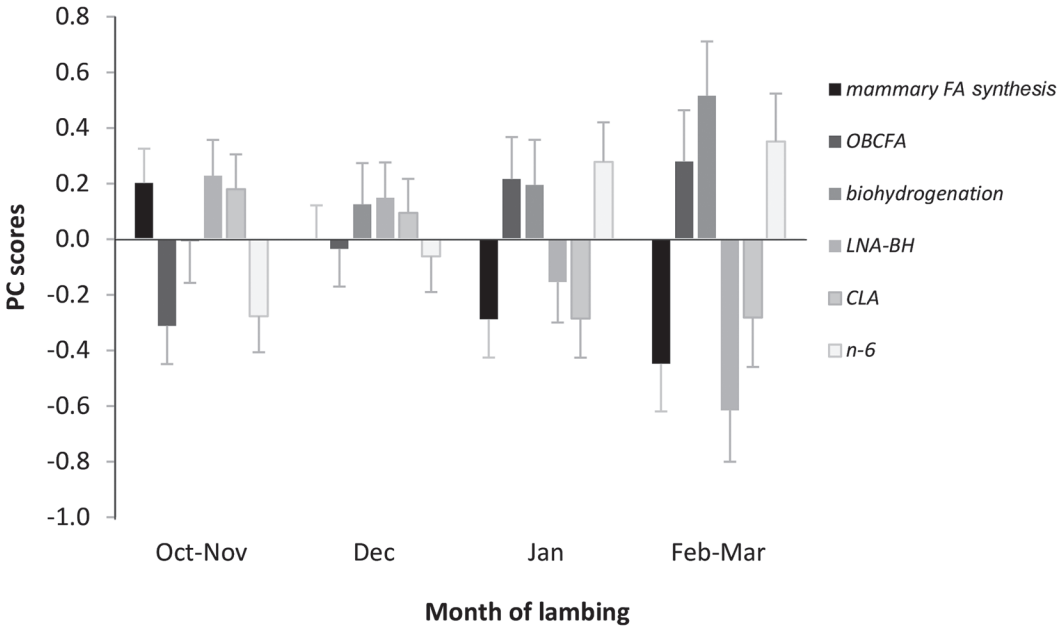


**Figure 8.** Least squares means of mammary fatty acid synthesis, odd- and branched-chain fatty acid (OBCFA), biohydrogenation, linolenic acid biohydrogenation (LNA-BH), CLA, and n-6 factor scores for different lambing months. PC = principal component. Errors bars indicate SEM.

**Table 6.** Correlation matrix between the scores of principal components (PC) and latent factors

| Factor[1] | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 |
|---|---|---|---|---|---|---|---|---|---|
| F1: Mammary FA synthesis | −0.78*** | −0.25*** | 0.24*** | 0.16*** | 0.43*** | −0.03$^{NS}$ | 0.23*** | 0.05$^{NS}$ | 0.11*** |
| F2: OBCFA | 0.41*** | 0.21*** | 0.45*** | 0.51*** | 0.37*** | −0.36*** | −0.07* | 0.18*** | 0.13*** |
| F3: Biohydrogenation | 0.12*** | 0.04$^{NS}$ | −0.76*** | 0.25*** | 0.25*** | −0.09** | 0.42*** | 0.16*** | 0.26*** |
| F4: LNA-BH | −0.32*** | 0.50*** | −0.20*** | 0.40*** | −0.05$^{NS}$ | 0.35*** | −0.43*** | 0.30*** | −0.23*** |
| F5: Desaturase | 0.03$^{NS}$ | −0.61*** | 0.01$^{NS}$ | 0.55*** | −0.51*** | −0.01$^{NS}$ | 0.04$^{NS}$ | 0.20*** | −0.13*** |
| F6: CLA | −0.26*** | 0.37*** | −0.02$^{NS}$ | 0.29*** | −0.42*** | −0.36*** | 0.11*** | −0.60*** | 0.22*** |
| F7: n-3 | 0.14*** | 0.26*** | 0.31*** | 0.12*** | −0.12*** | 0.59*** | 0.66*** | −0.03$^{NS}$ | −0.05$^{NS}$ |
| F8: n-6 | 0.18*** | −0.27*** | −0.10** | 0.28*** | 0.34*** | 0.45*** | −0.31*** | −0.62*** | 0.12*** |
| F9, — | −0.04$^{NS}$ | −0.01$^{NS}$ | 0.11*** | −0.09$^{NS}$ | −0.23*** | 0.24*** | −0.20*** | 0.26*** | 0.87*** |

[1]OBCFA = odd- and branched-chain fatty acids; BH = biohydrogenation; LNA-BH = linolenic acid biohydrogenation.
NS > 0.05; * < 0.05; ** < 0.01; *** < 0.001.

profile. On the other hand, in spite of the large number of starting variables, MFA was able to identify a clear structure of the extracted latent variables through the factor pattern rotation. In particular, the ability of this technique to group FA according to their function or metabolic origin was confirmed. In agreement with previous works carried out in other ruminant species, MFA identified key pathways of the milk FA metabolism, as mammary gland de novo synthesis, ruminal BH, desaturation performed by SCD enzyme, and rumen microbial activity, that control a relevant quota (80%) of the complex correlation pattern among individual FA.

Some partial concordances between the 2 techniques have been observed. Both PC1 and F1 were related to the FA of mammary origin, and the correlation between their scores (Table 6) was rather large (about −0.80). A latent variable related to mammary gland FA synthesis able to explain the largest amount of variance was also obtained in other studies (Mele et al., 2016; Palombo et al., 2020). These results suggest the hypothesis of a role of the main driving force in regulating milk FA (co)variance patterns for the mammary FA synthesis pathway. Other large correlations were observed between F9 and PC9 (−0.87), BH factor (F3), and PC3 (−0.76), n-3 factor (F7), and PC7 (−0.66). This amount of covariation among PC and factors arise from the fact that both techniques start from the factorization of the correlation matrix. On the other hand, differences still remain due to the different assumptions on the covariance of the system. This fact, together with the possibility of rotating the factor pattern to improve its interpretation, provides more power to the MFA in identifying the real dimensions of milk FA profile system.

Principal component analysis confirmed its ability in reducing the dimension of the system, but it was not able to efficiently discriminate observations. It has to be considered that the animal sample of the present study was taken from commercial flocks where no specific experimental treatments were applied. Previous studies where PCA was able to distinguish clusters of observations were usually feeding trials where experimental diets aimed at modifying milk FA composition were tested. These treatments may have therefore enhanced differences between animals and emphasized the clustering of observations in the PC space.

A major criticism of MFA is the indeterminacy of its solutions and the lack of robustness against outliers (Wang et al., 2017). However, it should be pointed out that the various studies on the use of MFA for analyzing milk FA, carried out in different species and under different experimental conditions, led to very similar results. Such a consistency across studies could be considered as a proof for the adequacy of the MFA model to fit the covariance structure of milk FA composition.

Individual scores of latent factors extracted from the correlation matrix of FA were able to discriminate cows farmed in herds with different feeding management (Mele et al., 2016). They could therefore be used as synthetic indicators of milk FA metabolism for management purposes. Moreover, genetic parameters of latent factors have been estimated in dairy cattle (Cecchinato et al., 2019). Some latent variables, such as the one related to the activity of the SCD factor, showed moderate heritability (0.31), thus suggesting a possible use of factor scores as novel phenotypes in breeding plans. Instead of being considered simple traits, factor scores should be regarded as aggregate phenotypes and their inclusion as breeding goals should be aimed at improving milk nutritional quality through the modification of specific metabolic pathways.

## CONCLUSIONS

The 2 multivariate statistical techniques used in this study were able to efficiently summarize the milk FA profile of sheep with a reduced number of new variables. However, due to the partitioning of the variance in a large number of extracted variables, PCA was not able to distinguish stratification in the considered sample of

animals. On the other hand, the MFA revealed the existence of latent factors controlling the correlation pattern of milk FA. In particular, some independent factors were associated with metabolic pathways involved in the synthesis and modification of milk FA, both in the mammary gland and in the rumen. Moreover, essential FA of dietary origin (PUFA n-3 and PUFA n-6) were associated with 2 independent factors, confirming diet as an important factor affecting milk FA profile. The results of the mixed linear model showed a weak influence of the fixed effects on the extracted factors. The clear meaning of the extracted latent factors suggests their possible role as novel phenotypes for breeding and management purposes.

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