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

Comparison between principal components and multivariate factor analysis to investigate detailed milk fatty acid profile in sheep.

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ABSTRACT

The fatty acid profile is one of the most important aspects of the nutritional properties of milk. Fatty acid content in milk is affected by several factors 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 fatty acid profile by extracting a reduced number of new variables. In this work, the milk fatty acid profile of a sample of 993 Sarda breed ewes was analysed with PCA and MFA in order to compare the ability of these two 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, type of lambing, altitude of flock location, and the random effect of flock nested within altitude. Both techniques extracted the same number of new variables (9) explaining 80% of the total variance. In general, PCA structures were difficult to interpret, with only four PC being associated to a clear meaning. PC1 in particular was the easier to interpret and agreed with the interpretation of the first factor, being both associated to the FA of mammary origin. On the other hand, MFA was able to identify a clear structure of 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 SCD enzyme, and rumen microbial activity, confirming previous findings in sheep and in other species. Generally, the new extracted variables were mainly affected by physiological factors as DIM, parity and lambing-month; the type of lambing had no effect on the new variables, altitude influenced only one PC and factor. Both techniques were able to summarize a larger amount of the original variance into a reduced number of variables. Moreover, factor analysis conformed its ability in identifying latent common factors clearly related to fatty acid metabolic pathways.

Keyword: fatty acids, principal components, factor analysis,

INTRODUCTION

The interest of the scientific community and of the 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, 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) being the diet one of the most important factors affecting milk FA profile (Nudda et al., 2014). However, other factors such as genetics (Carta et al., 2008; Correddu et al., 2019), physiology (De La Fuente et al., 2009), and environment (Sevi et al., 2002) can affect milk FA composition.

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 suggested for investigating such a complex correlation network. In particular, principal components analysis (**PCA**) (Fievez et al., 2003;

Kadegowda et al., 2008) and Multivariate Factor Analysis (**MFA**) (Conte et al., 2016; 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 two techniques. PCA is a model-free approach and it is mostly aimed at compressing the variance of the system. PCA is particularly useful when few PC can explain large portion of the variance. 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). MFA aims at investigating the covariance structure and, in particular, at identifying common latent variables (factors) that generate the quota of shared covariance among the original variables (Krzanowski, 2000; Morrison, 1976). In other words, PCA is more focused on the observations whereas MFA is on the variables, respectively.

PCA 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). PCA 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 (Caneque et al., 2014). MFA was successfully used to elucidate relationship between milk FA in dairy cows (Mele et al., 2016; Conte et al., 2016), Sheep (Palombo et al., 2020), and buffaloes (Correddu et al., 2017).

The use of the two 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 two new variables related to the whole lactation and to the shape

of lactation curve, respectively. On the same data, MFA generates two 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 the use of MFA and PCA in the analysis of milk FA profile in sheep, in order 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 (APA). FA profile of the milk samples was measured using gas chromatography (GC) as previously described (Correddu et al., 2017).

Statistical analysis

Data for a total of 49 individual FA were analyzed with PCA and MFA using SAS PRINCOMP and FACTOR procedures, respectively (SAS Inst. 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), 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.

Individual principal component and factor scores for each ewe were calculated and then analyzed with the following mixed linear model:

$$y_{ijklmno} = \mu + PAR_j + DIM_k + LM_l + LT_m + ALT_n + F(ALT)_o + e_{ijklmno}$$

where $y_{ijklmnop}$ was the principal component or factor score; *PAR* is the fixed effect of the *j*-th parity class (eight classes from 1 to >7); DIM is the fixed effect the *k*-th days in milking interval (five intervals: < 110, 110 to 140, 141 to 170, 171 to 200, >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); LT, is the fixed effect of the the *m*-th type of lambing (two classes: single and multiple birth); ALT, is the fixed effect of the *n*-th altitude of location of flocks (mountain > 500 mt above the sea level; hill ≤ 500 and ≥ 200 m a.s.l; plain < 200 m a.s.l.). Finally, *F(ALT)* is the random effect of the *o*-th flock nested within altitude of location; and $e_{ijklmno}$ is the residual term. No effect of the date of the test was included in the model because in most of flocks all samples were collected in the same day.

RESULTS AND DISCUSSION

Descriptive statistics of detailed milk FA composition of the 993 samples of sheep milk are reported in Supplementary Table 1.

Principal component analysis

Nine principal components (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. 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 two PC, even though an overlapped stratification according to parity (Figure 1a) or DIM class (Figure 1b) could be appreciated. In previous studies on milk FA, PCA was effective in grouping animals according to diet they were fed (Correddu et al., 2016; Bernard et al., 2009). PCA 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 (Caneque et al., 2004). Such a different discriminating power among studies could be ascribed to the amount of variance accounted for by the first two PC: 40% in the present study, and 90% in

127 the paper of Correddu et al. (2016), respectively. This is a consequence of the different number of
128 original variables considered (49 and 21, respectively). The larger number of original variables, and
129 therefore of extracted PC (equal to the number of original variables), resulted in the partition of the
130 total variance on a larger number of eigenvalues.

131 The analysis of eigenvector structure is a way for assigning a meaning to the extracted PC in
132 terms of relationship with the original variables. In the present study, the interpretation of the
133 extracted PC on the basis of their eigenvectors (Table 1) was rather difficult. Considering a threshold
134 of ≥ 0.20 (absolute value), half of the FA exhibited coefficients exceeding this value in at least
135 two/three different PC, whereas four FA showed no loading>0.20 for any extracted PCA (Table 1).
136 This was particularly true for PC4, PC5, PC7, and PC9. An interpretation was attempted for the other
137 PCs.

138 The first PC (PC1) presented highest loadings for most of the short and medium chain FA
139 (negatives), on some iso FA, C18:1*cis*-9 and long chain saturated FA (positives). Most of these FA
140 are totally or partially synthetized in the mammary gland (Chilliard et al., 2000). Therefore, PC1
141 could be considered an index of the activity of this organ. The PC2 had high negative loadings on
142 anteisoC13, C14:0, C16:0, C14:1*cis*-9, C16:1*cis*-9, C18:3n-6 and positives on some
143 biohydrogenation products and C18:3*n*-3. The association with FA of different origin and metabolic
144 pathways does not allow to assign a clear meaning to this PC. The only feature shared by FA
145 associated to this PC is their relationship with diet quality, especially with the use of grazing. In dairy
146 cattle (Fievez et al., 2003) the two first PC were mostly associated to FA belonging to four groups.
147 Two included FA that originate in the mammary gland from *de novo* synthesis or desaturase activity;
148 the other two consists of FA produced in the rumen from the biohydrogenation activity or from
149 microbial synthesis.

150 The PC3 presented high positive loadings for C15:0 and C17:0, and negative for several
151 positional isomers of trans C18:1 and on C181*cis*-12, respectively. This PC could be related to the
152 FA biohydrogenation processes occurring in the rumen (Shingfield et al., 2010). The PC3 had also

high loadings on some FA of microbial origin. The OBCFA profile has been proposed as useful tool to predict shifts in microbial population associated in particular with the diet (Vlaeminck et al., 2006). PC6 showed the largest loadings for PUFA*n*-3, C18:2*n*-6, C18:1*trans*-11, and C18:2*cis*-9,*trans*-11, i.e., the substrates (the first two) and products (the last two) of the ruminal FA biohydrogenation. Thus, based also on the opposite loading sign for substrates and products, PC6 could be considered as an indicator of PUFA ruminal biohydrogenation activity. The PC8 had large positive loadings on C14:0, C18:1*trans*-4, 18:1*trans*-16+*cis*-14, and negative on C16:1*trans*-9, C18:1*trans*-11, C18:2*n*-6, C18:2*cis*-9,*trans*-11, C20:3*n*-6 and C20:4*n*-6 (negatives). Considering the high loadings exhibited by PUFA*n*-6 and by the main products of the biohydrogenation of C18:2*n*-6 (C18:1*trans*-11 and C18:2*cis*-9,*trans*-11), this PCA could be interpreted as an indicator of PUFA*n*-6 in the diet.

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 (Kaiser MSA). This index estimates the decrease of partial correlations compared to Pearson correlations between the observed variables. In the present work, the MSA parameter was 0.75, close to the value of 0.80 indicated as the optimal threshold for the suitability of a dataset 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 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 to PC (Table 1).

The communality of original variables was on average 0.81 (±0.11), similar to the value reported for buffaloes (0.79) (Correddu et al., 2017) and higher than in cattle (0.69) (Conte et al., 2016; Mele et al., 2016). to 0.96 (for C10:0), The two FA with the lowest value of communality (0.54 for C18:2*trans*-9,*trans*-12 and C18:3*n*-6) were the same reported in a work on buffaloes (C18:2*trans*-9,*trans*-12 and C18:3*n*-6) . Therefore in both species these two FA are characterised by

about 50% of independent variation. Largest communalities, in agreement with previous studies, have been found for short and medium chain saturated FA (e.g.: C6:0, C8:0, C10:0, C12:0), associated to 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 to a factor (Macciotta et al., 2015).

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 saturated FA (C20:0, C22:0 and C24:0). Thus, it was considered an index of "*mammary gland activity*". 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). F1 structure partially agrees with previous studies where it was associated to mammary gland ability to maintain an optimal milk fat fluidity and to the FA neosynthesis (Conte et al., 2016; Correddu et al., 2017; Palombo et al., 2020). The negative loadings of F1 for long chain saturated FA (C20:0, C22:0 and C24:0) was not observed in previous studies. In a recent investigation on Comisana sheep, they were associated to a factor interpreted as 'Branched fatty acids metabolism' (Palombo et al., 2020). In cows they were associated to a different factor together with other saturated and unsaturated LCFA (Conte et al. 2016; Mele et al., 2016), whereas in buffaloes they characterized a specific factor (Correddu et al., 2017).

Being positively associated to 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 two distinct factors associated with OCFA and BCFA were found in cattle and buffaloes (Conte et al. 2016; Correddu et al., 2017). The relative milk concentration of these FA depends on the composition of the microbial population (Vlaemink 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 consequence, the correlation pattern of all OBCFA is similar, and the underling pathway of variation is summarized in one unique latent factor.

Factor three and four were positively associated with all isomers of C18:1 and C18:2 originating from the ruminal biohydrogenation (**BH**) of PUFA, with the exception of C18:1*trans*-11 (vaccenic acid) and C18:2*cis*-9,*trans*-11 (rumenic acid). In particular F3 was associated with *trans* isomer of C18:1 from the 4th to the 10th position, C18:1*cis*-12 and, to a lesser extent, to C18:2*trans*-9,*trans*-12. F4 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 (C18:3*n*-3, α-linolenic acid, LNA). Although it is very difficult to unequivocally ascertain the metabolic origin of a specific minor BH intermediate (Shingfield et al., 2010), the separation of these FA into two different latent factors can suggest different metabolic pathways underling the BH of PUFA. In particular, FA associated to the 3th factor are often produced in the rumen during the BH process of C18:2*cis*-9,*cis*-12 (C18:2*n*-6, linoleic acid) (Shingfield et al., 2010). This result is in agreement with a previous report in cattle where an association of C18:2*n*-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 to F3 and, consequently, we decide to assign the generic name of "*biohydrogenation*". Considering the association of C18:3*n*-3 and of some its ruminal BH intermediates with the F4, this factor was named "*LNA-BH*". Almost

231 all FA here found to be associated to F3 and F4 were found in a single latent factor, together with
232 vaccenic and rumenic acids, in previous studies on cattle, buffalo and sheep (Conte et al., 2016;
233 Correddu et al., 2017; Palombo et al., 2020).

234 The fifth latent factor was named "*desaturase*", being positively associated with some
235 products of Stearoyl Coenzyme-A Desaturase (SCD) activity (C12:1*cis*-9, C14:1*cis*-9, C16:1*cis*-9
236 and C17:1*cis*-9) and negatively with the preferred substrate of this enzyme (C18:0). The other SCD
237 products, C18:1*cis*-9 and C18:2*cis*-9,*trans*-11, were highly correlated with the 1st and 7th latent
238 factors, respectively. This result is in agreement with previous investigations in buffaloes (Correddu
239 et al., 2017) and, partially, in cattle (Conte et al., 2016, Mele et al., 2016), where the C17:1*cis*-9 was
240 not associated with the factor related to SCD activity, but with the same factor including C18:1*cis*-9.
241 Results of the present study are also in partial agreement with a previous report in sheep (Palombo et
242 la., 2020). However, in this study the C17:1*cis*-9 did not correlated with any factor. Interestingly,
243 *desaturase* factor presented high loading value for C4:0 (-0.63), differently to previous studies where
244 this FA was associated to a factor with C6:0 (Mele et al., 2016), or was not associated with any factor
245 (Conte et al., 2016; Correddu et al., 2017).

246 Factor six was named CLA as it showed large correlations with C18:2*cis*-9,*trans*-11 (rumenic
247 acid) and C18:1*trans*-11 (vaccenic acid). It was associated to synthesis of the most abundant and
248 important milk CLA isomer (C18:2*cis*-9,*trans*-11) operated by the SCD in mammary gland. Rumenic
249 and vaccenic acids are of great importance for the nutritional quality of milk (Banni et al., 2003) and
250 many researches have been aimed to find strategies for increasing their concentration (Chilliard et al.,
251 2001; Nudda et al., 2014). High *CLA* factor scores indicate milk characterized by high nutritional
252 value, probably related to sheep grazing high quality pasture. The partition of the SCD products into
253 three different factors is in agreement with the work of Mele et al. (2016), which explained this result
254 with the chain length and the unsaturation degree of the substrate on SCD activity. Conversely,
255 rumenic and vaccenic acids were associated to the biohydrogenation factor in Comisana sheep
256 (Palombo et al. (2020). In the present study also C16:1*trans*-9 was correlated to the *CLA* factor. A

similar result, even though to e lesser extent, was reported in Mele et al. (2016). In another work, it was correlated with the factor associated to the LCFA (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 of the PUFA*n*-6 family, respectively. The extraction of two different factors for PUFA*n*-3 and *n*-6 is in agreement with recent report of buffaloes (Correddu et al., 2017), whereas in cattle they were associated to 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 to the capacity to promote C18:3*n*-3 and C18:2*n*-6 elongation, or to differences in the dietary concentration of these two FA (Correddu et al., 2016). Although their milk concentration is not high (0.5% of total FA, *n*-3 + *n*-6 excluding C18:3*n*-3 and C18:2*n*-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 is considered important for good health and normal development in humans (Simopoulos, 2002). The ninth factor explained the 3% of the total variance and did not showed significant loading values.

Mixed model analysis

Results of the mixed-model analysis carried out on the individual scores of the nine PC and of the nine 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 values 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 as diet, climate and farming practices on ruminal microbial environment (Henderson et al., 2015), which, in turn, influences FA biohydrogenation process and the production of OBCFA. For similar reasons a low

282 contribution of flock for the PC8 variance was not expected, being this PC interpreted as an indicator
283 of PUFA*n*-6 in the diet.

284 The DIM class significantly affected e PC1, PC2, and PC9 (Table 3). LS means of PC1 scores
285 exhibited an increasing trend across lactation stages (Figure 2). This trend underlines a reduction in
286 de novo FA synthesis as the lactation proceeds (they have negative loadings) together with an increase
287 of C18:1*cis*-9 synthesis, in agreement with the reports of Timmen and Patton (1988). The same trend
288 could be observed for PC9, even if the loadings of this PC were very lower compared to PC1. PC2
289 showed an opposite pattern (Figure 2).

290 Parity affected significantly PC1, PC5, PC6, and PC8. First lambing ewes exhibited the largest
291 LSmean of PC1 scores (Table 4), that was statistically different from later parities. The PC5 scores
292 decreased across parities, even if with some fluctuations. Scores of PC6 decreased from the 1st to the
293 5th parity and then increased till the 7th; whereas PC8 showed the opposite behavior (Table 4).
294 Interestingly, the effect of parity on PC6 underline a high concentration of both n-3 and n-6 PUFA in
295 primiparous sheep, followed by a decrease in the intermediate parities and then by an increase in the
296 last parities. Similarly to other milk composition traits, FA are affected by parity due to changes in
297 energy and overall metabolism of the ewes as the lactation number proceeds (González-García et al.,
298 2015). Results of the present study partially agree with previous researches that found higher
299 proportions of more desirable FA in milk of first-parity compared to later parities both in sheep and
300 cows. (Mierlita et al., 2011; Bilal et al., 2014). The larger content of favorable FA especially in first
301 parity animals is conformed also pattern of PC8 scores (Table 4).

302 The lambing month significantly affected PC1, PC5, PC6, and PC9. Scores for all these PC,
303 except from PC6 (Figure 3), were negative from October to December and positive from January to
304 March. PC1 exhibited larger absolute values in comparison to PC5 and PC9. Altitude of location of
305 flock affected only PC9 scores, with a decreasing trend passing from plain to mountain. The lambing
306 type did not affect any of the 9 PC.

307

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 to 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 *biohydrogenation* factors exhibited high values of variance explained by the flock effect (0.49 and 0.53, respectively).

Lambing type 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 4). 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 to late lactation evidenced by F1 pattern (Figure 4) are in agreement with previous reports in buffaloes (Correddu et al., 2017). On 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 be also 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 days 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 activity*. Such a decreasing pattern underlined a higher activity of LNA ruminal biohydrogenation and of CLA synthesis (due to the increase of SCD substrate, C18:1*trans*-11) in the first part of lactation compared to the last part. This finding was in agreement to that observed for the

PC2, and it could be explained by the high content of C18:3*n*-3 in spring Mediterranean pastures (Cabiddu et al., 2005), that tends to decrease as in late spring-summer. The pattern of the *Desaturase* factor underlines an increasing SCD activity as the lactation proceeds, as observed in cattle and buffaloes (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. An 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 an increase of FA produced by the ruminal microorganism, in particular by cellulolytic bacteria (Vlaemink et al., 2006). Higher scores for BCFA factor were observed in cows fed a diet with higher percentage of forage (Conte et al., 2016).

Parity had significant effect on *mammary activity*, *OBCFA*, *n-3*, and *n-6*. *Mammary activity* exhibited an increasing trend from 1st to third parity (Table 5) and then decreased till the eight parity. *OBCFA* scores were rather constant from the 1st to the 4th parity and then rapidly decrease in the 7th and 8th 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), others 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 to pluriparous animals, that exhibit 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 buffaloes (Mele et al., 2016; Correddu et al., 2017).

The month of lambing influenced significantly ($P<0.05$) all the latent factors, except from *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 3). An opposite trend could be observed for *OBCFA*, *biohydrogenation*, 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 season, and parity. Pluriparous ewes lamb in late fall-early winter, whereas first parity animals lamb in late winter-early spring. All the animals are then dried off at the beginning of summer. As a consequence, the number of autumn lambing ewes is larger, and they have also 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 the higher activity of 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 requirement. The lower scores of *LNA-BH* and *CLA* factors observed in milk of sheep lambing in winter underlines a lower activity of rumen LNA biohydrogenation, that result in low milk contents of alpha-linolenic acid, its biohydrogenation 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 to 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 two techniques

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

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

398 In general, PCA structures were difficult to interpret, also in comparison with previous
399 researches on milk FA profile. On the other hand, in spite of the large number of starting variables,
400 MFA was able to identify through the factor pattern rotation a clear structure of the extracted latent
401 variables. In particular, it was confirmed the ability of this technique, to group FA according to their
402 function or metabolic origin. In agreement with previous works carried out also in other ruminant
403 species, MFA identified key pathways of the milk FA metabolism, as mammary gland de novo
404 synthesis, ruminal biohydrogenation, desaturation performed by SCD enzyme, and rumen microbial
405 activity, that control a relevant quota (80%) of the complex correlation pattern among individual FA.

406 Some partial concordances between the two techniques have been observed. Both PC1 and F1
407 were related to the FA of mammary origin, and the correlation between their scores (Table 6) was
408 rather large (about -0.80). A latent variable related to mammary gland activity able to explain the
409 largest amount of variance was obtained also in other studies (Mele et al., 2016; Palombo et al., 2020).

These results suggest to hypothesize a role of main driving force in regulating milk FA (co)variance pattern for mammary FA synthesis pathway. Other large correlations were observed between F9 and PC9 (-0.87), *Biohydrogenation* factor and PC3 (-0.76), *n-3* factor and PC7 (-0.66). This amount of covariation among principal components 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.

PCA 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 emphasised the clustering of observations in the PC space.

A major criticism to MFA is for the indeterminacy of its solutions and for 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 analysing 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 la., 2016). They could be therefore 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, 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 two 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 multivariate factor analysis revealed the existence of latent factors controlling the correlation pattern of milk fatty acids. In particular, some independent factors were associated to 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 to two independent factors, confirming the diet as important factor in 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 suggest to hypothesise a possible role as novel phenotypes for breeding and management purposes.

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573

Supplementary Table 1. Descriptive statistic for individual fatty acids in sheep fat milk (n = 993)

Descrizione	Mean	SD	CV(%)	Min	Max
C4:0	2.67	0.37	13.83	1.52	4.05
C6:0	1.75	0.37	21.02	0.46	2.65
C8:0	1.60	0.46	28.46	0.28	2.84
C10:0	5.52	1.76	31.86	0.87	10.18
C10:1	0.02	0.01	51.71	0.00	0.06
C11:0	0.25	0.09	34.30	0.05	0.65
C12:0	3.48	1.00	28.78	1.08	8.15
iso C13:0	0.03	0.01	34.04	0.01	0.08
C12:1	0.04	0.01	33.41	0.02	0.13
iso C14:0	0.13	0.04	33.41	0.04	0.33
C14:0	10.81	1.54	14.23	5.28	18.42
iso C15:0	0.31	0.07	23.79	0.11	0.66
anteiso C15:0	0.54	0.11	20.81	0.21	0.91
C14:1c9	0.20	0.08	42.43	0.04	0.68
C15:0	1.17	0.18	15.36	0.57	2.37
iso C16:0	0.34	0.07	20.73	0.08	0.65
C16:0	25.95	2.97	11.43	18.51	36.69
iso C17:0	0.44	0.09	19.99	0.14	0.80
C16:1*trans*-9	0.20	0.10	48.97	0.06	0.73
anteiso C17:0	0.49	0.08	17.19	0.15	0.78
C16:1cis-9	0.89	0.26	29.01	0.41	2.30
C17:0	0.78	0.11	14.46	0.42	1.32
C17:1cis-9	0.23	0.06	25.30	0.11	0.61
C18:0	10.29	2.51	24.38	1.37	21.00
C18:1*trans*-4	0.02	0.01	49.99	0.00	0.16
C18:1*trans*-5	0.02	0.01	53.52	0.00	0.12
C18:1*trans*-6 + 8	0.23	0.11	49.45	0.07	1.10
C18:1*trans*-9	0.27	0.08	31.56	0.13	0.91
C18:1*trans*-10	0.42	0.44	105.73	0.11	7.85
C18:1*trans*-11	2.06	1.03	50.21	0.46	5.77
C18:1*trans*-13 + *trans*-14	0.86	0.45	51.90	0.22	4.74
C18:1c9	17.23	3.64	21.11	5.37	34.75
C18:1cis-12	0.31	0.13	40.17	0.11	1.07
C18:1*trans*-16 + c14	0.50	0.15	29.34	0.12	1.08
C18:2*trans*-9,*trans*-12	0.02	0.01	63.00	0.01	0.18
C18:2cis-9,*trans*-13	0.44	0.17	38.08	0.14	1.64
C18:2cis-9,*trans*-12	0.15	0.03	23.38	0.07	0.34
C18:2n6	2.09	0.51	24.33	0.92	4.32
C20:0	0.32	0.12	39.19	0.04	1.36
C18:3n6	0.04	0.02	39.81	0.01	0.15
C18:3n3	0.89	0.50	55.76	0.20	3.35
C18:2cis-9,*trans*-11	1.03	0.47	45.52	0.28	3.16
C22:0	0.17	0.06	32.76	0.02	0.50
C20:3n6	0.03	0.01	29.32	0.01	0.07
C20:4n6	0.13	0.05	36.62	0.04	0.33
EPA	0.06	0.02	29.91	0.03	0.15
C24:0	0.08	0.03	40.23	0.00	0.19
DPA	0.13	0.03	27.05	0.04	0.28
DHA	0.04	0.02	38.70	0.01	0.12

576 **Table 1**. Eigenvectors and eigenvalues of the first nine principal components extracted from the
577 correlation matrix of the 49 Fatty acids.

	Principal Component (PC)								
	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
isoC13:0	0.192	0.019	0.113	-0.114	-0.042	-0.072	0.099	0.137	0.143
anteisoC13:0	-0.094	**-0.246**	0.071	**0.217**	-0.029	-0.051	0.096	0.181	-0.032
isoC14:0	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
isoC15:0	**0.210**	0.044	0.134	0.030	0.024	**-0.213**	0.033	0.063	0.004
anteisoC15:0	0.090	0.128	0.198	0.193	0.101	**-0.278**	-0.057	0.041	-0.027
C14:1cis-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
isoC16:0	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**
isoC17:0	**0.214**	0.035	-0.035	0.092	0.183	-0.131	0.032	0.025	**-0.207**
C16:1trans-9	-0.114	**0.213**	0.023	0.106	**-0.202**	-0.180	0.077	**-0.311**	0.147
anteisoC17:0	0.127	0.105	0.096	**0.241**	**0.249**	-0.148	-0.060	-0.014	**-0.211**
C16:1cis-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
C17:1cis-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
C18:1trans-4	0.096	0.030	**-0.246**	-0.041	0.107	-0.015	**0.245**	**0.202**	0.147
C18:1trans-5	0.054	0.027	**-0.263**	0.031	0.119	0.007	**0.274**	0.117	0.185
C18:1trans-6+8	0.030	0.038	**-0.344**	0.106	0.060	-0.087	0.147	0.056	0.116
C18:1trans-9	0.025	0.064	**-0.339**	0.107	0.002	-0.121	0.121	0.008	0.067
C18:1trans-10	-0.007	-0.013	**-0.245**	0.194	0.086	-0.003	0.093	-0.066	0.131
C18:1trans-11	-0.122	**0.233**	-0.033	0.104	-0.138	**-0.214**	0.081	**-0.263**	0.186
C18:1trans-13+t14	-0.154	**0.216**	-0.080	0.125	0.088	0.117	-0.154	0.156	0.001
C18:1cis-9	**0.229**	-0.018	-0.089	-0.012	-0.100	-0.030	-0.012	-0.059	**-0.336**
C18:1cis-12	0.071	-0.043	**-0.294**	0.095	0.126	0.089	-0.090	0.032	0.037
C18:1trans-16+cis-14	-0.090	**0.284**	-0.073	0.056	0.064	0.117	-0.160	**0.210**	-0.128
C18:2trans-9,trans-12	-0.030	0.013	-0.159	**0.253**	0.033	0.152	0.031	0.001	**0.205**
C18:2cis-9,trans-13	-0.139	0.162	-0.101	**0.253**	-0.091	0.119	-0.166	0.124	-0.174
C18:2cis-9trans-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
C18:2cis-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
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**
DPA	0.090	0.137	0.150	0.064	-0.069	**0.299**	**0.367**	-0.072	-0.087
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
Var. explained (%)	25.06	15.06	13.37	7.83	5.32	5.27	3.13	2.89	2.57

578

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

	Factors[1]									Com[2]
	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
isoC13:0	-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
anteisoC15:0	-0.08	**0.86**	-0.19	0.01	-0.06	0.20	0.01	-0.14	-0.13	0.85
isoC16:0	-0.20	**0.81**	-0.03	-0.05	-0.02	-0.06	-0.04	0.16	0.06	0.73
anteisoC17:0	-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
isoC14:0	-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
isoC15:0	-0.47	**0.66**	-0.08	-0.34	-0.02	-0.07	0.07	-0.12	-0.07	0.81
isoC17:0	-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
C18:3*n*-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
DPA	-0.20	0.17	-0.12	0.03	-0.08	0.04	**0.88**	0.03	-0.05	0.87
DHA	-0.25	0.07	-0.04	-0.11	0.02	-0.15	**0.77**	0.12	-0.03	0.71
EPA	0.11	0.09	-0.23	0.27	-0.10	0.20	**0.75**	-0.12	0.07	0.78
C18:2*n*-6	-0.20	0.06	0.10	0.14	0.06	-0.13	0.06	**0.80**	0.13	0.76
C20:4*n*-6	-0.18	0.12	0.12	-0.39	0.13	-0.25	0.13	**0.67**	-0.24	0.81
C20:3*n*-6	-0.18	0.17	0.20	-0.28	0.07	-0.21	0.07	**0.66**	-0.13	0.68
C18:3*n*-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	
Var. explained (%)	17.62	10.80	9.46	9.36	9.29	6.86	6.00	5.54	3.01	

[1] F1 = *Mammary activity*; F2 = *OBCFA*; F3= *Biohydrogenation*; F4 = *LNA (alpha-linolenic acid) BH*; F5 = *Desaturase*; F6 = *CLA*; F7 = *n-3*; F8 = *n-6*; F9 = *C16*.

[2] Communality.

Table 3. Effect of DIM, parity, month and type of lambing, and altitude of flock on the 9 principal components (PC) and 9 latent factors (F)

item		*P-value*					*Flock (zone)*
		DIM	Parity	Lambing-month	Lambing-type	Altitude	
Principal components							
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 factors[1]							
F1	*mammary activity*	**<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]Flock(zone) = contribute of flock nested within altitude of location to the total variance;
[2]*OBCFA* = odd and branched-chain fatty acids; *LNA-BH* = alpha-linolenic acid (C18:3*cis*-9,*cis*-12,*cis*-15) biohydrogenation; *CLA* = conjugated linoleic acids; *n-3* = polyunsaturated fatty acids belonging to the omega-3 family; *n-6* = polyunsaturated fatty acids belonging to the omega-6 family; *C16* = palmitic acid (C16:0).

Table 4. Least square means (± standard error) of the principal components affected by parity.

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

^{a,b,c,} least square means with different superscript letters within a column differ (P<0.05)

*although PC5 was significantly affect by parity, differences among contrasts did not reach the statistical significance ($\alpha = 0.05$).

Table 5. Least square means (± standard error) of the latent factors affected by parity

parity	*Latent factors*			
	mammary activity	*OBCFA*	*n-3*	*n-6*
1	-0.37^{b}±0.13	0.23^{ab}±0.14	0.09^{ab}±0.14	0.35^{a}±0.14
2	-0.06^{ab}±0.13	0.15^{ab}±0.15	-0.03^{abc}±0.14	0.11^{ab}±0.14
3	0.04^{a}±0.13	0.23^{a}±0.14	-0.24^{c}±0.14	0.08^{ab}±0.13
4	-0.04^{ab}±0.13	0.21^{a}±0.14	-0.21^{bc}±0.14	-0.07^{b}±0.13
5	-0.08^{ab}±0.13	0.08^{abc}±0.15	-0.15^{abc}±0.14	-0.05^{b}±0.14
6	-0.10^{ab}±0.14	-0.01^{abc}±0.15	0.05^{a}±0.15	0.01^{ab}±0.14
7	-0.16^{ab}±0.15	-0.15^{bc}±0.16	0.06^{abc}±0.16	0.15^{ab}±0.15
8	-0.29^{ab}±0.20	-0.45^{c}±0.21	-0.14^{abc}±0.20	-0.01^{ab}±0.20

^{a,b,c,} least square means with different superscript letters within a column differ (P<0.05)

Table 6 Correlation matrix between the scores of principal components and latent factors

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Factor1 Mammary	-0.78	-0.25	0.24	0.16	0.43	-0.03	0.23	0.05	0.11
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3976	<0.0001	0.1532	0.0008
Factor2 OBCFA	0.41	0.21	0.45	0.51	0.37	-0.36	-0.07	0.18	0.13
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0276	<0.0001	<0.0001
Factor3 BH	0.12	0.04	-0.76	0.25	0.25	-0.09	0.42	0.16	0.26
	0.0002	0.2467	<0.0001	<0.0001	<0.0001	0.0067	<0.0001	<0.0001	<0.0001
Factor4 LNA BH	-0.32	0.50	-0.20	0.40	-0.05	0.35	-0.43	0.30	-0.23
	<0.0001	<0.0001	<0.0001	<0.0001	0.1463	<0.0001	<0.0001	<0.0001	<0.0001
Factor5 Desaturase	0.03	-0.61	0.01	0.55	-0.51	-0.01	0.04	0.20	-0.13
	0.3162	<0.0001	0.7817	<0.0001	<0.0001	0.8356	0.234	<0.0001	<0.0001
Factor6 CLA	-0.26	0.37	-0.02	0.29	-0.42	-0.36	0.11	-0.60	0.22
	<0.0001	<0.0001	0.5706	<0.0001	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
Factor7 N3	0.14	0.26	0.31	0.12	-0.12	0.59	0.66	-0.03	-0.05
	<0.0001	<0.0001	<0.0001	0.0001	0.0003	<0.0001	<0.0001	0.3595	0.1358
Factor8 N6	0.18	-0.27	-0.10	0.28	0.34	0.45	-0.31	-0.62	0.12
	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
Factor9	-0.04	-0.01	0.11	-0.09	-0.23	0.24	-0.20	0.26	0.87
	0.2497	0.671	0.0004	0.0072	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Figure Captions

Figure 1. Plots of the scores for the first two principal components (PC1 and PC2) of animals belonging to different class of DIM (from 1 to 5 in figure 1A and averaged in mid and late lactation in figure 2B).

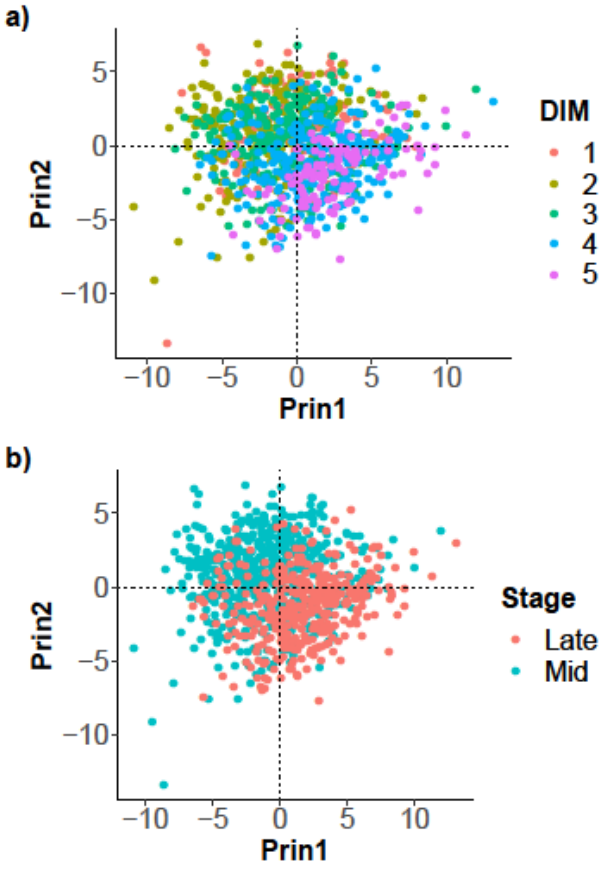
Figure 2. Classes of days in milk (DIM) pattern of PC1, PC2 and PC9.

Figure 3. Effect of lambing month on PC1, PC5, PC6 and PC9.

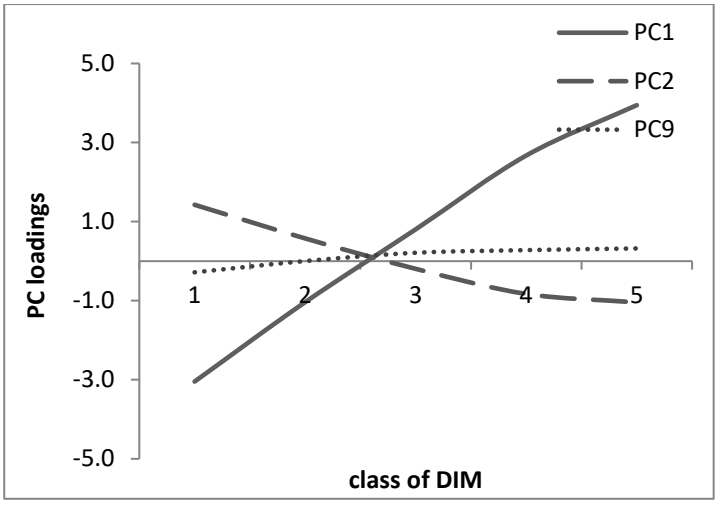
Figure 4. Classes of days in milk (DIM) pattern of *mammary activity*, *OBCFA*, *LNA-BH*, *Desaturase* and *CLA* factors.

Figure 5. Effect of lambing month on *mammary activity*, *OBCFA*, biohydrogenation, *LNA-BH*, *CLA* and *n-6* factors.

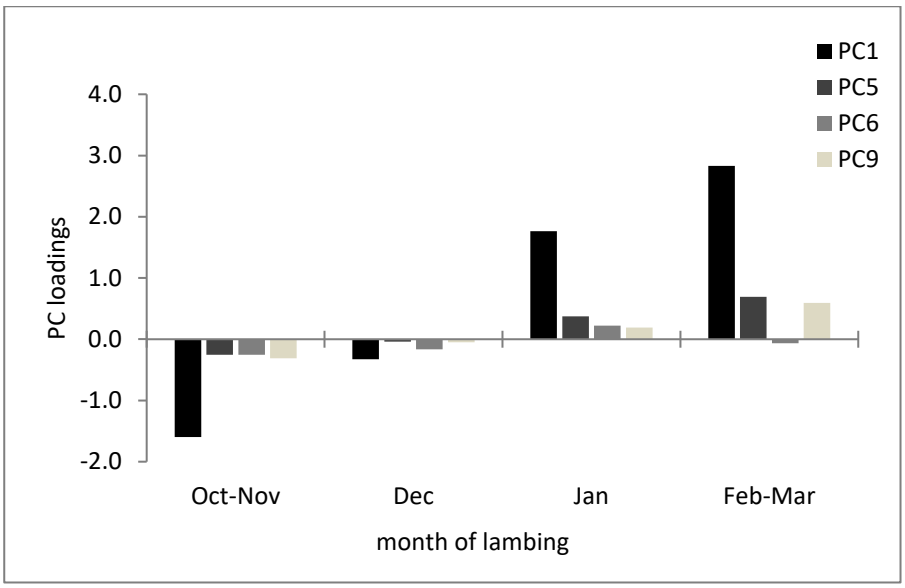
Correddu. Figure 1.



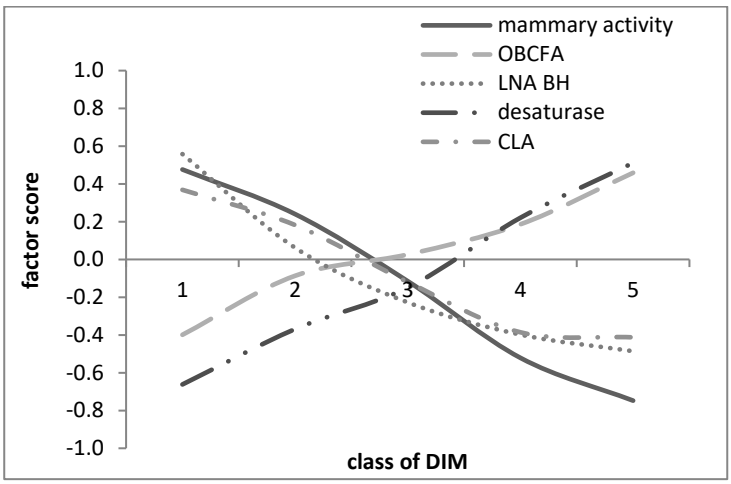
Correddu. Figure 2.



Correddu. Figure 3.



650 **Correddu. Figure 4.**
651



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Correddu. Figure 5.