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

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25 April 2024

1 **Comparison between principal components and multivariate factor analysis to investigate**

2 **detailed milk fatty acid profile in sheep.**

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10 **ABSTRACT**

11   The fatty acid profile is one of the most important aspects of the nutritional properties of milk.

12 Fatty acid content in milk is affected by several factors as diet, physiology, environment, and genetics.

13 Recently, Principal Component Analysis (PCA) and Multivariate Factor Analysis (MFA) have been

14 used to summarize the complex correlation pattern of the milk fatty acid profile by extracting a

15 reduced number of new variables. In this work, the milk fatty acid profile of a sample of 993 Sarda

16 breed ewes was analysed with PCA and MFA in order to compare the ability of these two multivariate

17 statistical techniques in investigating the possible existence of latent substructures, and in studying

18 the influence of physiological and environmental effects on the new extracted variables. Individual

19 scores of PCA and MFA were analyzed with a mixed model that included the fixed effects of parity,

20 days in milking, lambing month, type of lambing, altitude of flock location, and the random effect of

21 flock nested within altitude. Both techniques extracted the same number of new variables (9)

22 explaining 80% of the total variance. In general, PCA structures were difficult to interpret, with only

23 four PC being associated to a clear meaning. PC1 in particular was the easier to interpret and agreed

24 with the interpretation of the first factor, being both associated to the FA of mammary origin. On the

25 other hand, MFA was able to identify a clear structure of all the extracted latent variables, confirming

26 the ability of this technique, to group FA according to their function or metabolic origin. Key

27 pathways of the milk FA metabolism were identified, as mammary gland *de novo* synthesis, ruminal

28 biohydrogenation, desaturation performed by SCD enzyme, and rumen microbial activity, confirming

29 previous findings in sheep and in other species. Generally, the new extracted variables were mainly

30 affected by physiological factors as DIM, parity and lambing-month; the type of lambing had no

31 effect on the new variables, altitude influenced only one PC and factor. Both techniques were able to

32 summarize a larger amount of the original variance into a reduced number of variables. Moreover,

33 factor analysis conformed its ability in identifying latent common factors clearly related to fatty acid

34 metabolic pathways.

35

36 **Keyword**: fatty acids, principal components, factor analysis,

37

38 **INTRODUCTION**

39 The interest of the scientific community and of the consumers in the nutritional and health-

40 related properties of milk and dairy products has increased over the last decades. Strategies for

41 improving the milk content of some categories of fatty acids (**FA**) considered beneficial for human

42 health, as PUFA and CLA, have been developed. Most of them rely on feeding management,

43 (Dewhurst et al., 2006; Toral et al., 2010; Nudda et al., 2014) being the diet one of the most important

44 factors affecting milk FA profile (Nudda et al., 2014). However, other factors such as genetics (Carta

45 et al., 2008; Correddu et al., 2019), physiology (De La Fuente et al., 2009), and environment (Sevi et

46 al., 2002) can affect milk FA composition.

47 The elucidation of FA metabolic pathways and the knowledge of factors affecting their

48 regulation are of great interest for improving milk nutritional properties. In particular, the complex

49 phenotypic and genetic correlation pattern existing among individual milk FA hampers the

50 modification of FA profile via feeding and genetic strategies (Cecchinato et al., 2019). Dimension-

51 reduction multivariate statistical methods have been suggested for investigating such a complex

52 correlation network. In particular, principal components analysis (**PCA**) (Fievez et al., 2003;

53 Kadegowda et al., 2008) and Multivariate Factor Analysis (**MFA**) (Conte et al., 2016; Mele et al.,

54 2016; Correddu et al., 2017; Palombo et al., 2020) have been used to highlight common metabolic

55 pathways of FA in ruminant species.

56   Being both based on the factorization of the covariance or correlation matrix, and on the

57 representation of the multivariate system with a lower number of new variables, PCA and MFA

58 appear somewhat similar. However, the way the factorization is carried out differs between the two

59 techniques. PCA is a model-free approach and it is mostly aimed at compressing the variance of the

60 system. PCA is particularly useful when few PC can explain large portion of the variance. On the

61 other hand, MFA starts from a model of the covariance structure of the multivariate system. In

62 particular, the factor model assumes that the covariance of a system could be partitioned in a

63 component shared by all the variables (communality) plus a component specific of each variable

64 (uniqueness). MFA aims at investigating the covariance structure and, in particular, at identifying

65 common latent variables (factors) that generate the quota of shared covariance among the original

66 variables (Krzanowski, 2000; Morrison, 1976). In other words, PCA is more focused on the

67 observations whereas MFA is on the variables, respectively.

68   PCA of cattle milk FA composition was able to assess the relationship between individual

69 milk FA and diet-induced milk fat depression (Kadegowda et al., 2008), and to investigate metabolic

70 relationships among milk FA and to describe their origin (Fievez et al., 2003). PCA has been also

71 used to analyze meat FA profile to differentiate lamb meat according to their origin (Díaz et al., 2005),

72 and to study the relationship between quality traits of carcass and meat of light lamb (Caneque et al.,

73 2014). MFA was successfully used to elucidate relationship between milk FA in dairy cows (Mele et

74 al., 2016; Conte et al., 2016), Sheep (Palombo et al., 2020), and buffaloes (Correddu et al., 2017).

75   The use of the two methods on the same data may provide different and complementary

76 results. In a study of cattle lactation curve traits, for example, PCA was able to extract from the

77 correlation matrix of test day records two new variables related to the whole lactation and to the shape

78 of lactation curve, respectively. On the same data, MFA generates two latent factors related to the

79 first and the second part of lactation, respectively (Macciotta et al., 2006).

80   The aim of this work was to compare results of the use of MFA and PCA in the analysis of

81 milk FA profile in sheep, in order to assess their ability to investigate the complex correlation pattern

82 that exists among these variables.

83

84 <div align="center">**MATERIALS AND METHODS**</div>

85 *Animals and milk samples*

86   The study was carried out on individual milk samples of 993 Sarda dairy ewes farmed in 48

87 flocks located in the island of Sardinia (Italy). Individual milk samples (one per sheep) were collected

88 from April to July 2014, during the morning milking, by the Provincial Association of Animal

89 Breeders (APA). FA profile of the milk samples was measured using gas chromatography (GC) as

90 previously described (Correddu et al., 2017).

91 *Statistical analysis*

92   Data for a total of 49 individual FA were analyzed with PCA and MFA using SAS

93 PRINCOMP and FACTOR procedures, respectively (SAS Inst. Inc., Cary, NC). The number of

94 principal components (PC) to retain was defined according to the amount of explained variance ($\geq$

95 80%). In MFA, the number of factors to be extracted was based on their eigenvalue (>1), on their

96 readability in terms of relationships with the original variables and biological meaning, and on the

97 amount of explained variance. Factor interpretation was improved through a VARIMAX rotation.

98   Individual principal component and factor scores for each ewe were calculated and then

99 analyzed with the following mixed linear model:

100 $$y_{ijklmno} = \mu + PAR_j + DIM_k + LM_l + LT_m + ALT_n + F(ALT)_o + e_{ijklmno}$$

101 where $y_{ijklmnop}$ was the principal component or factor score; *PAR* is the fixed effect of the *j*-th parity

102 class (eight classes from 1 to >7); DIM is the fixed effect the *k*-th days in milking interval (five

103 intervals: < 110, 110 to 140, 141 to 170, 171 to 200, >200); LM is the fixed effect of the *l*-th class of

104 lambing month (1: January; 2: February and March; 3: October and November; 4: December); LT, is

105 the fixed effect of the the *m*-th type of lambing (two classes: single and multiple birth); ALT, is the

106 fixed effect of the *n*-th altitude of location of flocks (mountain > 500 mt above the sea level; hill $\leq$

107 500 and $\geq$ 200 m a.s.l; plain < 200 m a.s.l.). Finally, *F(ALT)* is the random effect of the *o*-th flock

108 nested within altitude of location; and $e_{ijklmno}$ is the residual term. No effect of the date of the test was

109 included in the model because in most of flocks all samples were collected in the same day.

110

111   **RESULTS AND DISCUSSION**

112   Descriptive statistics of detailed milk FA composition of the 993 samples of sheep milk are

113 reported in Supplementary Table 1.

114

115 ***Principal component analysis***

116   Nine principal components (PC) were able to explain about 80% of the total variance of the

117 system. (Table 1). The variance explained ranged from about 25% for PC1 to about 3% for PC9,

118 respectively. PC scores are often used in dispersion plots to highlight possible clustering or trends in

119 the observations. In the present work, no clear clustering of observations has been detected in the

120 space of the first two PC, even though an overlapped stratification according to parity (Figure 1a) or

121 DIM class (Figure 1b) could be appreciated. In previous studies on milk FA, PCA was effective in

122 grouping animals according to diet they were fed (Correddu et al., 2016; Bernard et al., 2009). PCA

123 was also applied on lamb meat FA to differentiate animals according to their geographical origin

124 (Díaz et al., 2005), or to study the relationship between quality traits of carcass and meat of light

125 lambs (Caneque et al., 2004). Such a different discriminating power among studies could be ascribed

126 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, C18:1cis-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, C14:1cis-9, C16:1cis-9, C18:3n-6 and positives on some

143   biohydrogenation products and C18:3n-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 C181cis-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

153   high loadings on some FA of microbial origin. The OBCFA profile has been proposed as useful tool

154   to predict shifts in microbial population associated in particular with the diet (Vlaeminck et al., 2006).

155   PC6 showed the largest loadings for PUFA*n*-3, C18:2*n*-6, C18:1*trans*-11, and C18:2*cis*-9,*trans*-11,

156   i.e., the substrates (the first two) and products (the last two) of the ruminal FA biohydrogenation.

157   Thus, based also on the opposite loading sign for substrates and products, PC6 could be considered

158   as an indicator of PUFA ruminal biohydrogenation activity. The PC8 had large positive loadings on

159   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,

160   C18:2*cis*-9,*trans*-11, C20:3*n*-6 and C20:4*n*-6 (negatives). Considering the high loadings exhibited by

161   PUFA*n*-6 and by the main products of the biohydrogenation of C18:2*n*-6 (C18:1*trans*-11 and

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

163

164   *Factor analysis*

165   The suitability of the data set to the theoretical assumptions of the MFA was assessed through

166   the calculation of the Kaiser Measure of Sampling Adequacy (Kaiser MSA). This index estimates the

167   decrease of partial correlations compared to Pearson correlations between the observed variables. In

168   the present work, the MSA parameter was 0.75, close to the value of 0.80 indicated as the optimal

169   threshold for the suitability of a dataset to MFA (Cerny and Kaiser, 1977). This result was similar to

170   previous reports on the use of MFA on milk FA profile (Mele et al., 2016; Correddu et al., 2017).

171   Nine factors able to explain about 80% of the total variance of the system were extracted (Table 2).

172   The pattern of explained variance across the different factors was smoother compared to PC (Table

173   1).

174   The communality of original variables was on average 0.81 (±0.11), similar to the value

175   reported for buffaloes (0.79) (Correddu et al., 2017) and higher than in cattle (0.69) (Conte et al.,

176   2016; Mele et al., 2016). to 0.96 (for C10:0), The two FA with the lowest value of communality

177   (0.54 for C18:2*trans*-9,*trans*-12 and C18:3*n*-6) were the same reported in a work on buffaloes

178   (C18:2*trans*-9,*trans*-12 and C18:3*n*-6) . Therefore in both species these two FA are characterised by

179   about 50% of independent variation. Largest communalities, in agreement with previous studies, have

180   been found for short and medium chain saturated FA (e.g.: C6:0, C8:0, C10:0, C12:0), associated to

181   the first or second latent factor. The high values observed for these FA, and the agreement among

182   studies, confirm that the variability of these FA is mostly related to a unique metabolic pathway,

183   similar among species.

184   The adequateness of the factor model for fitting the FA correlation matrix was confirmed by

185   the simple structure of the rotated pattern (Morrison, 1976). In particular, each factor showed large

186   loadings with few variables and small loadings with the other variables (Table 2), respectively. Each

187   variable had a large loading in only one factor, with only one exception (C16:0). In total, 42 out of

188   49 FA exhibited a loading value ≥0.60, considered as an empirical threshold for declaring a variable

189   associated to a factor (Macciotta et al., 2015).

190   The first latent factor (F1) was positively correlated with short and medium chain FA (apart

191   from C4:0 and C16:0) and negatively with C18:1*cis*-9 and some long chain saturated FA (C20:0,

192   C22:0 and C24:0). Thus, it was considered an index of "*mammary gland activity*". A peculiarity of

193   F1 is its structural similarity with PC1. A concordance between the results of the first PC and the first

194   factor extracted from the same data set was observed in a study on body conformation traits in cows

195   (Olasege et al., 2019). F1 structure partially agrees with previous studies where it was associated to

196   mammary gland ability to maintain an optimal milk fat fluidity and to the FA neosynthesis (Conte et

197   al., 2016; Correddu et al., 2017; Palombo et al., 2020). The negative loadings of F1 for long chain

198   saturated FA (C20:0, C22:0 and C24:0) was not observed in previous studies. In a recent investigation

199   on Comisana sheep, they were associated to a factor interpreted as 'Branched fatty acids metabolism'

200   (Palombo et al., 2020). In cows they were associated to a different factor together with other saturated

201   and unsaturated LCFA (Conte et al. 2016; Mele et al., 2016), whereas in buffaloes they characterized

202   a specific factor (Correddu et al., 2017).

203   Being positively associated to the odd, iso, and anteiso FA (except iso C13:0), F2 was named

204   "*OBCFA*". These FA are almost completely synthesized by rumen microorganisms (Vlaeminck et

205 al., 2006). This result is in agreement with a previous report on sheep (Palombo et al., 2020), whereas

206 two distinct factors associated with OCFA and BCFA were found in cattle and buffaloes (Conte et al.

207 2016; Correddu et al., 2017). The relative milk concentration of these FA depends on the composition

208 of the microbial population (Vlaemink et al., 2006). The diet, especially its forage to concentrate

209 ratio, is one of the main factors affecting the relative abundance of microbial populations. Thus,

210 feeding management could affect the proportions of OCFA and BCFA in milk. Sheep involved in the

211 present study are farmed in the typical Mediterranean semi-extensive systems with pasture as main

212 feeding source (Macciotta et al., 1999; Molle et al., 2007). Under these conditions, forage to

213 concentrate ratio in the diet should be approximately similar in the various flocks and, therefore, also

214 the rumen microbial composition to a certain extent. As consequence, the correlation pattern of all

215 OBCFA is similar, and the underling pathway of variation is summarized in one unique latent factor.

216 Factor three and four were positively associated with all isomers of C18:1 and C18:2

217 originating from the ruminal biohydrogenation (**BH**) of PUFA, with the exception of C18:1*trans*-11

218 (vaccenic acid) and C18:2*cis*-9,*trans*-11 (rumenic acid). In particular F3 was associated with *trans*

219 isomer of C18:1 from the 4th to the 10th position, C18:1*cis*-12 and, to a lesser extent, to C18:2*trans*-

220 9,*trans*-12. F4 was associated with *trans* isomer of C18:1 from the 13th to the 16th position, C18:2*cis*-

221 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).

222 Although it is very difficult to unequivocally ascertain the metabolic origin of a specific minor BH

223 intermediate (Shingfield et al., 2010), the separation of these FA into two different latent factors can

224 suggest different metabolic pathways underling the BH of PUFA. In particular, FA associated to the

225 3th factor are often produced in the rumen during the BH process of C18:2*cis*-9,*cis*-12 (C18:2*n*-6,

226 linoleic acid) (Shingfield et al., 2010). This result is in agreement with a previous report in cattle

227 where an association of C18:2*n*-6 and its intermediate products in the same latent factor was found

228 (Mele et al., 2016). In the present study C18:2*cis*-9,*cis*-12 was not associated to F3 and, consequently,

229 we decide to assign the generic name of "*biohydrogenation*". Considering the association of C18:3*n*-

230 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

257   similar result, even though to e lesser extent, was reported in Mele et al. (2016). In another work, it

258   was correlated with the factor associated to the LCFA (Conte et al., 2016).

259   The seventh and eighth latent factors were named "*n-3*" and "*n-6*" as they were positively

260   correlated with FA of the PUFA*n-3* family and of the PUFA*n-6* family, respectively. The extraction

261   of two different factors for PUFA*n-3* and *n*-6 is in agreement with recent report of buffaloes

262   (Correddu et al., 2017), whereas in cattle they were associated to a unique latent factor (Conte et al.,

263   2016; Mele et al., 2016). This result could arise from differences in the metabolism of these FA, in

264   particular to the capacity to promote C18:3*n*-3 and C18:2*n*-6 elongation, or to differences in the

265   dietary concentration of these two FA (Correddu et al., 2016). Although their milk concentration is

266   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

267   nutritional importance (Connor, 2000). In particular high concentrations of PUFA along with a low

268   *n*-6 to *n*-3 ratio is considered important for good health and normal development in humans

269   (Simopoulos, 2002). The ninth factor explained the 3% of the total variance and did not showed

270   significant loading values.

271

272   **Mixed model analysis**

273   Results of the mixed-model analysis carried out on the individual scores of the nine PC and

274   of the nine extracted factors are reported in Table 3.

275

276   *Principal components*

277   On average, the contribution of the flock to the PC variance was around 46%, with the highest

278   values exhibited by PC3 (69%) and the lowest by PC8 (31%). The high contribution of the flock to

279   the variance of PC3 could arise from the great influence of environmental factors as diet, climate and

280   farming practices on ruminal microbial environment (Henderson et al., 2015), which, in turn,

281   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

308

309   Results of the mixed-model analysis factor scores are reported in Table 3. On average, the

310   contribution of the flock effect to the total variance was 45%, with the highest values for the *n-3*

311   (55%) and the lowest for the *desaturase* (25%) factors, respectively. This finding is consistent with

312   the larger effect of environmental and management factors on the milk content of FA arising from

313   the diet (i.e: PUFA) compared to those of endogen production (i.e.: MUFA produced by delta-9

314   desaturase) (Stoop et al., 2008; Correddu et al., 2019). According to the high value observed for PC3,

315   the *OBCFA* and *biohydrogenation* factors exhibited high values of variance explained by the flock

316   effect (0.49 and 0.53, respectively).

317   Lambing type and the altitude of flock location did not affect any of the extracted factors.

318   The DIM significantly affected *mammary activity*, *OBCFA*, *LNA-BH*, *desaturase*, and *CLA* factor

319   scores. In particular least squares means for scores of *mammary activity*, *LNA-BH* , and *CLA*

320   decreased along the lactation, whereas *OBCFA* and *desaturase* exhibited an opposite trend (Figure

321   4). The effect of DIM class on the *mammary activity* factor confirmed results obtained for PC1. The

322   higher contents of *de novo* FA and lower of C18:1*cis*-9 in early compared to late lactation evidenced

323   by F1 pattern (Figure 4) are in agreement with previous reports in buffaloes (Correddu et al., 2017).

324   On dairy cows a different behavior was observed (Conte et al., 2016; Mele et al., 2016). Such

325   differences could be partially ascribed to differences in the metabolism among species, even if the

326   data distribution along the lactation should be also considered. In the typical Mediterranean sheep

327   farming system, the milk of the first month of lactation is suckled by the lamb. Thus, milk tests

328   considered in the present work were available only from 45 days after parturition, The lack of data

329   for the first month could have therefore hampered the modeling of a trend of FA metabolic pathway

330   in early lactation. Lactation patterns of *LNA-BH* and *CLA* factors evidenced a trend similar to

331   *mammary gland activity*. Such a decreasing pattern underlined a higher activity of LNA ruminal

332   biohydrogenation and of CLA synthesis (due to the increase of SCD substrate, C18:1*trans*-11) in the

333   first part of lactation compared to the last part. This finding was in agreement to that observed for the

334 PC2, and it could be explained by the high content of C18:3n-3 in spring Mediterranean pastures

335 (Cabiddu et al., 2005), that tends to decrease as in late spring-summer. The pattern of the *Desaturase*

336 factor underlines an increasing SCD activity as the lactation proceeds, as observed in cattle and

337 buffaloes (Mele et al., 2016; Correddu et al., 2017). According to Mele et al. (2016), the increasing

338 trend of *OBCFA* factor along the lactation can be related to the variation of forage to concentrate

339 ratio. An higher amount of concentrate is usually provided in early lactation to meet energy needs of

340 the animals; as the lactation proceeds, there is an increase of the proportion of forages in the diet

341 resulting an increase of FA produced by the ruminal microorganism, in particular by cellulolytic

342 bacteria (Vlaemink et al., 2006). Higher scores for BCFA factor were observed in cows fed a diet

343 with higher percentage of forage (Conte et al., 2016).

344 Parity had significant effect on *mammary activity*, *OBCFA*, *n-3*, and *n-6*. *Mammary activity*

345 exhibited an increasing trend from 1st to third parity (Table 5) and then decreased till the eight parity.

346 *OBCFA* scores were rather constant from the 1st to the 4th parity and then rapidly decrease in the 7th

347 and 8th parities. The *n-3* and *n-6* factors showed a similar waving pattern (Table 5). There is a lack of

348 consensus on the effect parity on latent factors extracted from milk FA. Some works evidenced a

349 large effect (Mele et al., 2016), others minor or no effect (Conte et al., 2016; Correddu et al., 2017).

350 The effect of parity on milk FA is mainly due to the larger PUFA content in primiparous compared

351 to pluriparous animals, that exhibit higher amount of SFA. These figures have been observed both in

352 cows and sheep (Mierlita et al., 2011; Bilal et al., 2014). Differences between parities in the extent of

353 tissue mobilization and in the content of FA synthase in the mammary gland, as well as the rumen

354 microflora, can partially explain the effect of parity on milk FA (Miller et al., 2006; Friggens et al.,

355 2007). In the present work, first lambing animals exhibited lower scores for *mammary activity*, and

356 higher for *n-3* and *n-6* factors, respectively. Scores of the *OBCFA* factor underlined a decreasing

357 pattern of ruminal derived FA with age, as previously reported in cows and buffaloes (Mele et al.,

358 2016; Correddu et al., 2017).

359   The month of lambing influenced significantly (P<0.05) all the latent factors, except from

360   *desaturase* and *n-3*. *Mammary activity*, *LNA-BH*, and *CLA* factors exhibited positive scores for

361   lambings occurring from October to December and negative scores for those from January to March,

362   respectively (Figure 3). An opposite trend could be observed for *OBCFA*, *biohydrogenation*, and *n-*

363   *6*. Sheep lambing is strictly seasonal, thus the evaluation of the effect of lambing month on a

364   productive response has a different meaning in comparison, for example, with dairy cattle.

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

366   production season, and parity. Pluriparous ewes lamb in late fall-early winter, whereas first parity

367   animals lamb in late winter-early spring. All the animals are then dried off at the beginning of

368   summer. As a consequence, the number of autumn lambing ewes is larger, and they have also longer

369   lactations. Autumn lambing sheep were sampled in late-lactation, whereas winter lambing sheep were

370   sampled in mid-lactation. Thus, the effects on FA profile of the physiological condition of the animal

371   (stage of lactation, parity) and of the environment (mainly pasture quality) on the FA profile are

372   difficult to disentangle. For example, the larger scores for *mammary activity* found in autumn lambing

373   sheep reflect the higher activity of mammary gland in the FA synthesis in late lactation, whereas

374   winter lambing sheep showed higher content of FA derived from body reserve mobilization in early

375   lactation to meet energy requirement. The lower scores of *LNA-BH* and *CLA* factors observed in milk

376   of sheep lambing in winter underlines a lower activity of rumen LNA biohydrogenation, that result

377   in low milk contents of alpha-linolenic acid, its biohydrogenation intermediates, C18:1*trans*-11 and

378   C18:2*cis*-9,*trans*-11. This pattern reflects, probably, the lower quality of pastures in late spring

379   compared to late-winter early-spring. This finding has interesting implications on the quality of milk

380   in relationship to the season of lambing and to the availability of high-quality pasture, evidencing

381   higher content of desired FA in milk of sheep lambed in autumn.

382

383   **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).

410 These results suggest to hypothesize a role of main driving force in regulating milk FA (co)variance

411 pattern for mammary FA synthesis pathway. Other large correlations were observed between F9 and

412 PC9 (-0.87), *Biohydrogenation* factor and PC3 (-0.76), *n-3* factor and PC7 (-0.66). This amount of

413 covariation among principal components and factors arise from the fact that both techniques start

414 from the factorization of the correlation matrix. On the other hand, differences still remain due to the

415 different assumptions on the covariance of the system. This fact, together with the possibility of

416 rotating the factor pattern to improve its interpretation, provides more power to the MFA in

417 identifying the real dimensions of milk FA profile system.

418 PCA confirmed its ability in reducing the dimension of the system, but it was not able to

419 efficiently discriminate observations. It has to be considered that the animal sample of the present

420 study was taken from commercial flocks where no specific experimental treatments were applied.

421 Previous studies where PCA was able to distinguish clusters of observations were usually feeding

422 trials where experimental diets aimed at modifying milk FA composition were tested. These

423 treatments may have therefore enhanced differences between animals and emphasised the clustering

424 of observations in the PC space.

425 A major criticism to MFA is for the indeterminacy of its solutions and for the lack of

426 robustness against outliers (Wang et al., 2017). However, it should be pointed out that the various

427 studies on the use of MFA for analysing milk FA, carried out in different species, and under different

428 experimental conditions, led to very similar results. Such a consistency across studies could be

429 considered as a proof for the adequacy of the MFA model to fit the covariance structure of milk FA

430 composition.

431 Individual scores of latent factors extracted from the correlation matrix of FA were able to

432 discriminate cows farmed in herds with different feeding management (Mele et la., 2016). They could

433 be therefore used as synthetic indicators of milk FA metabolism for management purposes. Moreover,

434 genetic parameters of latent factors have been estimated in dairy cattle (Cecchinato et al., 2019). Some

435 latent variables, as the one related to the activity of the SCD factor, showed moderate heritability

436   (0.31), thus suggesting a possible use of factor scores as novel phenotypes in breeding plans. Instead

437   of being considered simple traits, factor scores should be regarded as aggregate phenotypes and their

438   inclusion as breeding goals should be aimed at improving milk nutritional quality through the

439   modification of specific metabolic pathways.

440

441

CONCLUSIONS

443   The two multivariate statistical techniques used in this study were able to efficiently summarize

444   the milk FA profile of sheep with a reduced number of new variables. However, due to the partitioning

445   of the variance in a large number of extracted variables, PCA was not able to distinguish stratification

446   in the considered sample of animals. On the other hand, the multivariate factor analysis revealed the

447   existence of latent factors controlling the correlation pattern of milk fatty acids. In particular, some

448   independent factors were associated to metabolic pathways involved in the synthesis and modification

449   of milk FA, both in the mammary gland and in the rumen. Moreover, essential FA of dietary origin

450   (PUFAn-3 and PUFAn-6) were associated to two independent factors, confirming the diet as

451   important factor in affecting milk FA profile. The results of the mixed linear model showed a weak

452   influence of the fixed effects on the extracted factors. The clear meaning of the extracted latent factors

453   suggest to hypothesise a possible role as novel phenotypes for breeding and management purposes.

454

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573

574 **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

575

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

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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	

580 [1] F1 = *Mammary activity*; F2 = *OBCFA*; F3= *Biohydrogenation*; F4 = *LNA (alpha-linolenic acid) BH*;

581 F5 = *Desaturase*; F6 = *CLA*; F7 = *n-3*; F8 = *n-6*; F9 = *C16*.

582 [2] Communality.

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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		DIM	Parity	Lambing-month	Lambing-type	Altitude	*Flock (zone)*
				P-value			
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*

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[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).

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

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

605 [a,b,c,] least square means with different superscript letters within a column differ (P<0.05)
606 *although PC5 was significantly affect by parity, differences among contrasts did not reach the
607 statistical significance ($\alpha = 0.05$).
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612 **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	$-0.37^b \pm 0.13$	$0.23^{ab} \pm 0.14$	$0.09^{ab} \pm 0.14$	$0.35^a \pm 0.14$
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	$-0.29^{ab} \pm 0.20$	$-0.45^c \pm 0.21$	$-0.14^{abc} \pm 0.20$	$-0.01^{ab} \pm 0.20$

613 [a,b,c,] least square means with different superscript letters within a column differ (P<0.05)

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619 **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

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621 **Figure Captions**
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624 **Figure 1.** Plots of the scores for the first two principal components (PC1 and PC2) of animals

625 belonging to different class of DIM (from 1 to 5 in figure 1A and averaged in mid and late lactation

626 in figure 2B).

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

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

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

630 *Desaturase* and *CLA* factors.

631 **Figure 5.** Effect of lambing month on *mammary activity*, *OBCFA*, biohydrogenation, *LNA-BH*,

632 *CLA* and *n-6* factors.

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634　**Correddu. Figure 1.**
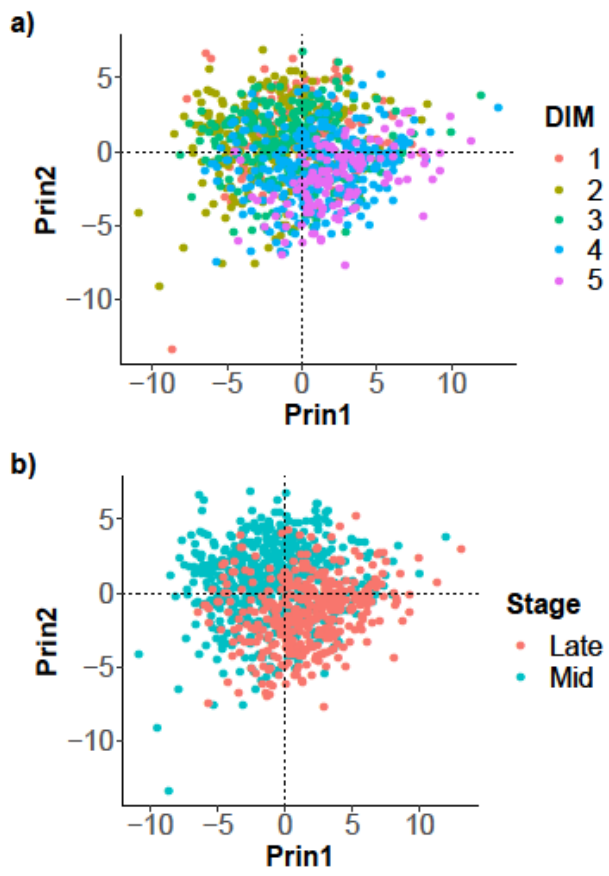
638 **Correddu. Figure 2.**
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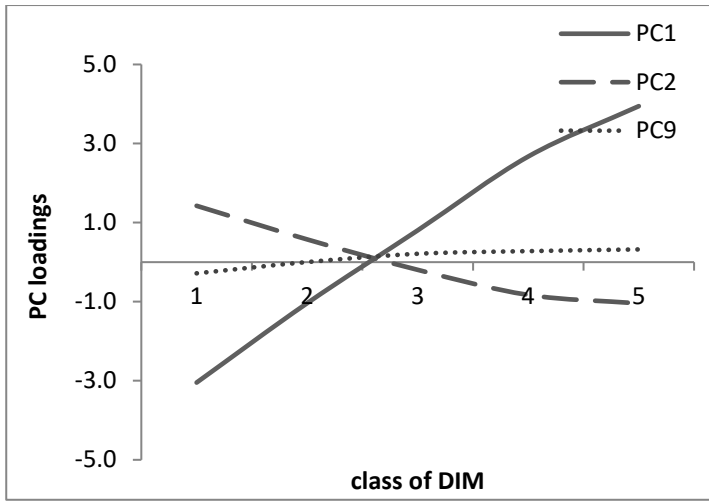
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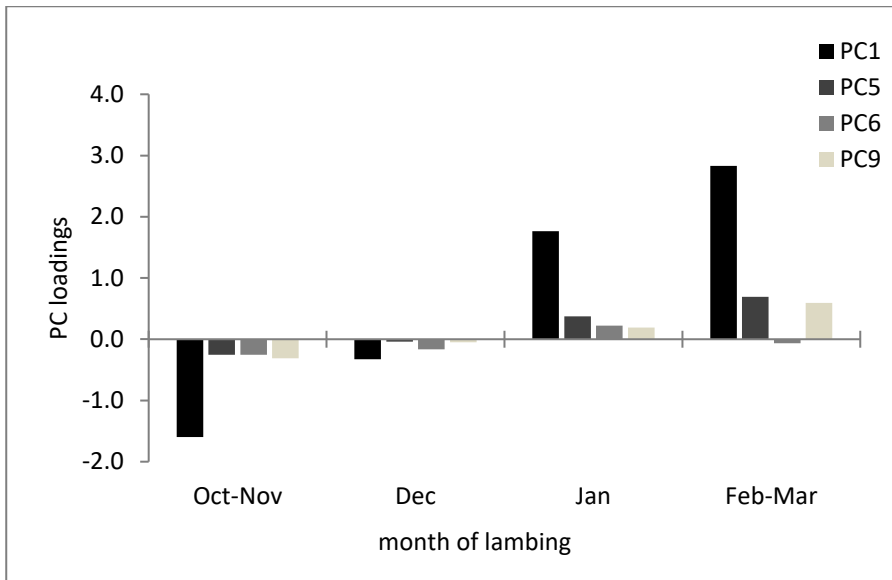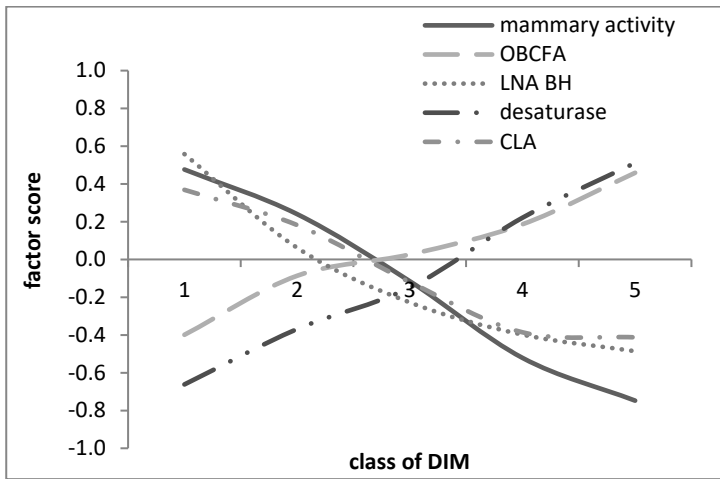645
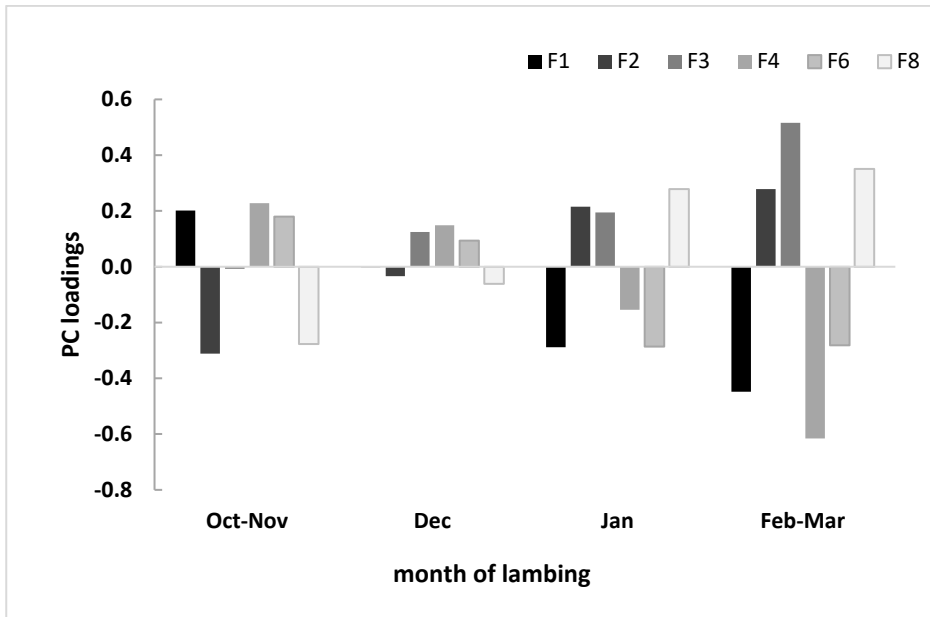646
647 **Correddu. Figure 3.**

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Correddu. Figure 4.

654 **Correddu. Figure 5.**
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