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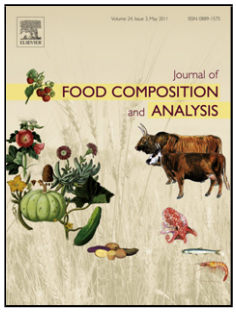

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Use of multivariate factor analysis to characterize the fatty acid profile of buffalo milk

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Highlights

Multivariate factor analysis was applied to the fatty acid profile of buffalo's milk

A small number of latent factors were extracted from a complex pattern of fatty acids

The extracted factors showed specific biological meanings

Factors can be used as new quantitative phenotypes

Abstract

The suitability of multivariate factor analysis (MFA) to extract a small number of latent variables able to explain the correlation pattern among fatty acids (FA) in buffalo milk was evaluated. FA profile of milk samples from 214 Italian water buffaloes was analysed by gas chromatography. MFA, performed on the correlation matrix of 52 FA, was able to extract 10 latent factors with specific biological meaning related to a common metabolic origin for FA associated with the same factor. Scores of the factors were treated as new quantitative phenotypes to evaluate the effect of age, month of calving and lactation stage. MFA approach was effective in describing the FA profile of buffalo milk by using a low number of new latent variables that clustered FA having similar metabolic origin and function. The new variables were also useful to test the effect of environmental and individual animal factors on milk FA composition.

Keywords: Buffalo's milk; Food composition; Food analysis; Milk fatty acids; Multivariate Factor Analysis; Lactation stage; De novo synthesis; Milk fat fluidity.

1. Introduction

Buffalo milk production (more than 100 million tonnes per year) represents 13.2% of milk produced worldwide, second after cow milk (83% with 638 million tonnes/year) (FAOSTAT, 2015). More than 97% of buffalo milk is produced in Asia. India and Pakistan are the largest producers. In Italy, the buffalo stock has increased from 185,000 in 2004 to 369,352 in 2014 (FAOSTAT, 2015). Almost all the milk (200,000 tonnes/year, FAOSTAT 2015) is processed into mozzarella cheese, a typical fresh cheese that has a high market value. For this reason the buffalo milk price is about three times that of milk of dairy cattle (Cipolat-Gotetet al., 2015).

Buffalo milk has a higher solids content compared to cow milk (Ahmad et al., 2008). In particular, milk fat content is rather high: it averaged 7.93% for Italian river buffaloes in 2015 (www.anasb.it). Fat content and its chemical composition play a crucial role in the definition of milk nutritional quality. In particular, the fatty acid (FA) profile is of great relevance for scientists, nutritionists and consumers, due to its effects on human health. Beneficial effects on human health have been reported for several milk FA. Some short-chain FA (SCFA), in particular C4:0 (butyric acid), which is found only in ruminant fat, is considered an important antineoplastic agent (Parodi, 1999); C18:1 *cis*-9 (oleic acid) and polyunsaturated FA (PUFA), particularly omega-3 PUFA, have favourable effects on risk factors for cardiovascular diseases and anti-inflammatory properties (Simopoulos, 2002; Baró et al., 2003). Potential beneficial effects on chronic diseases, such as cancer, atherosclerosis, obesity, bone density loss, and diabetes, have been reported for conjugated linoleic acid (CLA), in particular C18:2 *cis*-9,*trans*-11 (rumenic acid), (McGuire and McGuire, 2000; Banni et al., 2003), which derives from the ruminal biohydrogenation of C18:2 *cis*-9,*cis*-12 (C18:2 *n*–6, linoleic acid) and from the desaturation of C18:1 *trans*-11 (vaccenic acid), operated by stearoyl Co-A desaturase (SCD) enzyme in the mammary gland. Anticarcinogenic effect of C18:1 *trans*-11 has also been reported, depending on its conversion to C18:2 *cis*-9,*trans*-11 by SCD in human tissues (Lock et al., 2004).

A number of studies have been carried out to understand the factors affecting the FA composition of milk, with the aim of improving milk nutritional quality. Diet, breed, lactation stage, body condition, and environmental conditions have been reported as the main factors affecting the milk FA profile (Nudda et al., 2014). Studies on factors affecting buffalo milk FA composition reported effects of breed (Sun et al., 2014), roughage source (Penchev et al., 2016), flaxseed supplementation (Santillo et al., 2016), stage of lactation (Arumughan and Narayanan, 1981) and age (Qureshi et al., 2015), whereas the effect of the SCD genotype has been assessed, so far, only on a limited number of samples (Pauciullo et al., 2010).

Technology currently available for milk FA analysis allows the detection of a large number of molecules that can be classified into different groups: i) branched-chain FA, ii) *cis* and *trans* isomers of 18:1, 18:2 and 18:3, which are related to rumen activity, iii) *de novo* FA, which are synthesized in the mammary gland, iv) and other FA such as long-chain PUFA which derive from elongation process of FA arriving from diet or fat depots in the mammary gland.

Due to the high number of milk FA and the correlations between them, the FA profile represents a complex pattern to describe and understand. Multivariate factor analysis (MFA) is particularly suitable for studying and interpreting complex multivariate systems through the extraction of few variables (latent factors) with clear technical and biological meaning (Conte et al., 2016; Manca et al., 2016; Mele et al., 2016). Furthermore factor scores could be used as new phenotypes for further analyses.

The aims of this study were to evaluate the suitability of MFA in identifying a small number of latent factors allowing to interpret the relationship among FA in buffalo milk, and to study the effects of age of animals, month of calving and stage of lactation on the extracted factors.

2. Material and Methods

2.1. Milk sampling and fatty acid analysis

The study was carried out on individual milk samples of 214 Italian water buffaloes farmed in 13 herds located in Campania. Herds were characterized by a similar feeding management, based on 60% forage (silage and hay) and 40% concentrates. Sampling was carried out between 2011 and 2012, in collaboration with the Italian National Association of Buffalo Breeders (ANASB). The range of DIM in which animals were sampled was 2 to 345 (142.18 ± 74.75; mean ± SD).

The lipid extraction and the fatty acid methyl esters (FAME) preparation were performed as previously described (Nudda et al., 2005). The FAME were separated using a gas chromatograph (Turbo 3400 CX; Varian Inc., Palo Alto, CA), equipped with a capillary column (CP-select CB for FAME; 100 m × 0.32 mm i.d., 0.25 μm film thickness; Varian Inc.), a flame ionization detector (FID) and an automatic injector 8200 CX (Varian Inc.). Chromatographic conditions were set according to Correddu et al. (2016). The FAME peaks were routinely identified by comparing their retention times with those of authentic lipid standards and with published studies, as detailed in Nudda et al. (2005). Varian Star 3.4.1 software was used to compute the retention time and area of each individual FAME. FA were reported as g/100 g of total FAME.

2.2. Statistical analysis

The analysis of descriptive statistics and correlation coefficients of the 52 individual FA was carried out with MEAN and CORR procedures of SAS (SAS Inst. Inc., Cary, NC). In order to test the adequacy of data sets used for the factor analysis, the Kaiser Measure of Sampling Adequacy (Kaiser MSA) was calculated. This parameter summarizes the difference between Pearson and partial correlations. The correlation matrix was used to carry out an MFA, by using the FACTOR procedure of SAS. The number of factors to be extracted was based on their eigenvalue (>1), their readability in terms of relationships with the original variables, and on the amount of explained

variance. Factor readability was improved through a VARIMAX rotation. According to Macciotta et al. (2015), a variable was considered to be associated to a specific factor if the absolute value of its loading was ≥ 0.60.

Individual factor scores were then calculated and analysed with the following mixed linear model:

$$y_{ijklm} = \mu + age_i + month\ of\ calving_j + DIM_k + HTD_l + e_{ijklm}$$

where y_{ijkl} is the observed trait (i.e, the factor scores); μ is the overall mean; age_i is the fixed effect of the i^{th} age (i = 1 to 6); $month\ of\ calving_j$ is the fixed effect of the j^{th} month of calving (j = 1 to 12; January to December); DIM_k is the fixed effect of the k^{th} stage of lactation (k = 1 to 10; 30-d interval of DIM); HTD_l is the random effect of the l^{h} herd-test date (l = 29) $\sim N(0, \sigma^2_{HTD})$.; and e_{ijklo} is the random residual term $\sim N(0, \sigma^2_e)$.

3. Results and Discussion

3.1. Milk FA composition

Descriptive statistics of the milk FA profile are reported in Table 1. A total of 72 FA were identified but only those with mean and 5th percentile higher than 0.01% and zero, respectively, were considered. The FA profile comprised 22 SFA, 17 monounsaturated FA (MUFA) and 13 PUFA. The most abundant FA were palmitic acid (C16:0), oleic acid (C18:1 *cis*-9), myristic acid (C14:0), stearic acid (C18:0) and butyric acid (C4:0) which represented, together, 80% of the total FA. These results were consistent with previous works investigating the milk FA profile of Italian water buffalo (Varricchio et al., 2007; Blasi et al., 2008; Ménard et al., 2010). Although the values of SFA, MUFA and PUFA were similar to those observed in other ruminant species, differences in the individual FA composition could be observed. For example, C16:0, C18:0 and C14:0 account for 80% of the total SFA, whereas the contribution of short chain FA (C8:0, C10:0 and C12:0) is rather

low compared to what observed in other ruminant species (Côrtes et al., 2010; Toral et al., 2010; Bernard et al., 2015). An interesting result could be observed for C18:1 *trans*-11 and CLA *cis*-9,*trans*-11. Their averages were 0.91% and 0.41% of total FA, respectively; these values are markedly lower than those reported by Menard et al. (2010), e.g., 2.00% and 0.90% for C18:1 *trans*-11 and CLA *cis*-9,*trans*-11, respectively. On the other hand, our results are in agreement with a recent study on Mediterranean buffalo, that reported a similar value for CLA *cis*-9,*trans*-11 (0.45%) and slightly higher for C18:1 *trans*-11 (1.87%) (Pegolo et al., 2017). In the present study, the PUFA *n*–6 to PUFA *n*–3 ratio (*n*–6/*n*–3) ratio was very similar to the value reported by Santillo et al. (2016) for buffaloes fed with a diet including a moderate flaxseed supplementation (5.95 *vs* 5.76, respectively). Pegolo et al. (2017) reported a lower value (3.89). Such a difference could be ascribed to the C18:3 *n*–3 (α-linolenic acid) content. Different values for linoleic acid and α-linolenic acid were reported in previous studies (Varricchio et al., 2007; Blasi et al., 2008; Menard et al. 2010), resulting in high variability of the PUFA *n*–6 to PUFA *n*–3 ratio (*n*–6/*n*–3), that ranged from 1.3 to 10.0.

3.2. Multivariate factor analysis

Ten latent factors, able to explain about 80% of the total variance, were extracted by MFA from the FA correlation matrix (Table 2); C18:2 *n*–6 was excluded from the factor analysis due to its very low Kaiser value: 0.10. The explained variance was smoothly partitioned among factors, with factor 1 (F1) showing a small predominance (eigenvalue 7.57). This pattern of explained variance among extracted factors is peculiar of MFA (Conte et al., 2016). The Kaiser MSA was 0.77, close to 0.80 which is considered an empirical threshold that flags a dataset as suitable for MFA (Cerny and Kaiser, 1977). In total 40 out of 51 FA showed loading values ≥ 0.60 with only one factor. This result highlights a simple structure which represents an indicator for the suitability of the factor model assumption for the analysed data (Morrison, 1976). An exception is represented by C10:1 which exhibited loadings ≥ 0.60 for 2 factors.

F1 was positively correlated with short and medium chain FA, with the exception of C4:0, and negatively with C18:1 *cis*-9. These FA are *de novo* synthesized in the mammary gland from acetate by the acetyl CoA carboxylase and FA synthase enzymes (Chilliard et al., 2000). Moreover, C18:1 *cis*-9 is related to the activity of the SCD that catalyses desaturation of the C18:0 at the Δ9 position. Two recent works investigating the relationship of milk FA in cattle by MFA found a factor with a similar loading structure of F1 (Conte et al., 2016; Mele et al., 2016). The first latent factor was therefore associated with the mammary gland activity and, in particular, with the regulation of milk fat fluidity. The esterification of *de novo* short-chain FA (from 4 to 10 carbons) and 18:1 *cis*-9 at position *sn*-3 of glycerol plays a crucial role in the regulation of milk fat fluidity (Chilliard al., 2000). Interestingly, F1 showed opposite loadings for *de novo* FA and C18:1 *cis*-9, in agreement with previous reports on dairy cattle (Conte et al., 2016; Mele et al., 2016). Timmen and Patton (1988) proposed that the increase in milk C18:1 *cis*-9 due to the activity of SCD on C18:0 could be considered as a mechanism of milk fat fluidity maintenance when availability of *de novo* FA is reduced. This consideration is also confirmed, in the present study, by the inverse correlation found between the sum of concentrations of *de novo* FA and the concentration of C18:1 *cis*-9 (–0.78). For these reasons the first factor was named "*milk fat fluidity*".

The second latent factor (F2) was positively associated to almost all *cis* and *trans* isomers of C18:1, (except for *cis*-9, *cis*-11 and *trans*-4 isomers) and CLA *cis*-9,*trans*-11. Similar results were observed by Conte et al. (2016) in cattle. The FA associated with this factor share a common metabolic origin, being the intermediates of ruminal biohydrogenation of long-chain PUFA operated by rumen bacteria that leads to the formation of stearic acid (C18:0). The second factor was then named "*biohydrogenation*". C18:2 *n*–6 is often the most represented FA of dietary lipids in feed, in particular when the diet is rich in concentrates (feeds such as safflower, sunflower, soybean are rich in this FA). Milk of animals having large scores for biohydrogenation factor is richer in biohydrogenation products of C18:2 *n*–6. This condition may indicate intensive farm management,

with large use of concentrates and supplements. Results of the present study are in partial agreement with a previous report on Italian brown cows (Mele et al., 2016) where a latent factor highly correlated with intermediates of C18:2 *n*–6 biohydrogenation was obtained. However, in that work C18:1 *trans*-11 and CLA *cis*-9,*trans*-11 did not correlate with the factor associated with biohydrogenation products, as observed in the present work, but with a different factor, named CLA, considered an index of SCD activity in the mammary gland in converting part of C18:1 *trans*-11 into CLA *cis*-9,*trans*-11. On the other hand our results agreed with those of Conte et al. (2016) on dairy cattle.

The third extracted factor (F3) was named "*BCFA*", as it was positively correlated with branched-chain FA (BCFA). These FA in milk derive mainly from bacterial matter leaving the rumen. In particular, they are produced and used by rumen bacteria to maintain optimal fluidity of the microbial cell membrane (Vlaeminck et al., 2006). Growth and activity of ruminal microorganisms are affected by diet characteristics: type of forage, forage-to-concentrate ratio, lipid supplementation, and the presence of secondary plant metabolites. Thus the concentration and the relative abundance of BCFA in milk are affected by the diet (Vlaeminck et al., 2006; Correddu et al., 2015). Therefore BCFA concentration in milk could be used as a diagnostic tool to predict shifts in microbial population, mainly associated with the variation of diet composition (Vlaeminck et al., 2004; Cívico et al., 2017).

The fourth latent factor was positively associated with some long-chain saturated FA ("*LCSFA"*) C20:0, C22:0 and C24:0, and with C20:3 *n*–9. These FA may derive either from diet and body fat mobilization. In previous works on dairy cows these LCSFA were associated with a single latent factor together with *n*–6 and *n*–3 LCFA (Mele et al., 2016).

The fifth latent factor (F5) was positively associated with the C10:1 *cis*-9, C14:1 *cis*-9, C16:1 *cis*-9 and, to a lesser extent, with C17:1 *cis*-9 (loading value of 0.55). These monounsaturated FA (MUFA) are the products of the activity of SCD on their correspondent saturated substrates.

Consistently, this factor was negatively associated with the C18:0, which represents the preferred substrate for the activity of the SCD in mammary gland (Ntambi, 1999). It was interpreted as SCD activity and then named "*desaturase*" factor. However, not all products of SCD activity were represented in *desaturase*: in our study C18:1 *cis*-9 and C18:2 *cis*-9,*trans*-11 were associated with *milk fat fluidity* and *biohydrogenation*, respectively. This is not surprising as these FA are both involved in different metabolic pathways, such as ruminal biohydrogenation, mammary gland desaturase activity and milk fat fluidity regulation. Furthermore, our finding was in agreement with previous reports in dairy cattle (Conte et al., 2016; Mele et al., 2016). The diet, particularly its lipid content, is one of the major factors affecting SCD activity (Ntambi and Miyazaki, 2004; Bernard et al., 2013). Fernandes et al. (2007) reported a higher SCD activity in buffaloes fed on pasture compared to those fed concentrates. This result was explained by the higher level of C18:3 *n*–3 in pasture (which is partially biohydrogented to C18:1 *trans*-11, a substrate for SCD), compared with concentrates (rich in C18:2 *n*–6). Consistently, in sheep, the increased concentration of CLA in animals fed forage-based diets was associated with an increase in substrate availability (C18:1 *trans*-11), due to the high amount of C18:3 *n*–3 in the diet (Daniel et al., 2004).

The sixth and eighth latent factors (F6 and F8) were named "*n–6*" and "*n–3*" as they were positively associated with essential FA belonging to the family of omega-6 (C20:3 *n*–6, and to a lesser extent C20:2 *n*–6, C20:4 *n*–6) and omega-3 (C20:5 *n*–3, eicosapentaenoic acid, EPA; C18:3 *n*–3; C22:5 *n*–3, docosapentaenoic acid, DPA), respectively. The extraction of two different factors associated with *n*–6 and *n*–3 FA disagrees with previous works, where these FA were highly correlated with a single latent factor (Conte et al., 2016; Mele et al., 2016). Results of the present work suggest a different metabolism of these FA families in milk. In particular, different scores for these factors may categorize animals on the basis of their capacity to promote the elongation of C18:2 *n*–6 or C18:3 *n*–3. Milk concentration of *n*–6 and *n*–3 FA could be also related to differences in concentration of C18:2 *n*–6 or C18:3 *n*–3 in the diet of animals, thus allowing discrimination of

animals fed different diets or reared in different systems (intensive or extensive). An investigation on milk FA composition from sheep fed diets rich in C18:2 *n*–6 or C18:3 *n*–3 using principal component analysis highlighted an opposite sign of eigenvector coefficients for PUFA *n*–6 and PUFA *n*–3 in the same principal component that was named "*n*–6 to *n*–3 ratio" (Correddu et al., 2016). Similarly, the use of the C18:2 *n*–6 to C18:3 *n*–3 ratio has proven to be very effective in the discrimination of dairy goat fed diets supplemented with different lipid sources (Marín et al., 2012). The *n*–6 factor was negatively correlated with C16:0, differently from the work of Conte et al. (2016) where this FA was associated with the "biohydrogenation" factor, and from that of Mele et al. (2016) where C16:0 did not correlate with any latent factor.

The seventh latent factor (F7) was named "*C16:1 cis-7*" as it was positively correlated with this FA and, to a lesser extent, with C17:0 and C18:1 *cis*-11 (loadings 0.55 and 0.51, respectively). The meaning of this factor appears unclear, as these FA seem to have no common metabolic pathway. The origin of these FA is related to microbial synthesis (Lipski et al., 2001). The ninth latent factor (F9) was named "*OCFA*", due to its positive correlation with C13:0 and C15:0 (odd chain FA, OCFA). The extraction of two different factors correlated to OCFA and BCFA highlights differences in their metabolic pathway. Although both of them are either of microbial origin or *de novo* synthesized in the mammary gland, OCFA derives mainly from amylolytic whereas BCFA derives manly from cellulolytic bacteria respectively. Moreover, their *de novo* synthesis in the mammary gland starts from different substrates (Vlaeminck et al., 2006). F10 was named "*γ-linolenic acid*" because it was positively correlated only with this FA. C18:3 *n*–6 (γ-linolenic acid) is used by the body as a precursor for prostaglandin synthesis (Chapkin and Carmichael, 1990). Moreover, some beneficial properties for health have been reported, such as anti-inflammatory and anti-proliferative activities (Fan and Chapkin, 1998).

3.3. Effect of age, month of calving and stage of lactation on latent factors

Table 3 shows the effects of age, month of calving and DIM on the 10 extracted factors.

The age of animals significantly affected only the *OCFA* factor ($p < 0.05$). The scores of this factor were negative from age 1 to 4 (values: –0.58, –0.61, –0.32 and –0.29, respectively), increased to reach positive values at age 5 (0.12), then decreased from age 5 to 6 (0.12 and –0.63; $p < 0.05$). The trend of this factor with age suggests an increasing capacity of animals to produce OCFA, which drastically decreases in very old individuals.

The month of calving significantly affected *milk fat fluidity* ($p < 0.01$) and *desaturase* ($p < 0.05$) factors. *Milk fat fluidity* factor presented positive scores for animals that calved in January and August, whereas it presented negative scores in the other months of calving (Table 4). The trend of the scores during the month of calving increased slowly from February to August, then decreased rapidly from August to November, increased from December to January (reaching positive values) and decreased from January to February. It is worth noticing that buffaloes with positive scores for *milk fat fluidity* (Table 4) calved in January and August, and were sampled from November to January, i.e. in a short day period. On the other hand, buffaloes calving in February, November and December were sampled in June and July, i.e. on a long day period and exhibited the largest negative scores for this factor. Among environmental factors that affect milk FA composition, photoperiod can play an important role. Molik et al. (2011) reported an increase of *de novo* FA and a decrease of C18:1 (mainly of the *cis*-9 isomer) concentrations in sheep subjected to a short day period. These results are in agreement with our findings, because animals sampled in a short day period showed positive scores for *milk fat fluidity* which is correlated positively with *de novo* FA and negatively with C18:1 *cis*-9, respectively. *Desaturase* factor showed positive scores for animals that calved from January to April and negative scores for animals that calved from May to December (Table 4). In particular, significant differences were found between March and September ($p < 0.05$); in this case a photoperiod effect should be excluded, as almost all the animals included in these classes of calving were sampled in a same day period (November).

The effect of DIM was significant for *milk fat fluidity* ($p < 0.05$), *BCFA* ($p < 0.05$), *LCFA* ($p < 0.01$), *desaturase* ($p < 0.05$), and *C16:1 cis-7* ($p < 0.05$) factors. These factors were all associated with the activity of different mammary gland enzymes (*de novo* FA, elongase, desaturase), except for *BCFA* factor, which was also related to the activity of ruminal environment. However, the scores of these factors in the DIM showed different trends.

Milk fat fluidity factor (Figure 1) showed positive scores in the first part of lactation (from DIM 1 to 3), and negatives scores in middle and late lactation, reaching very negative values at DIM 9 and 10 (DIM > 240 days). Since this factor was associated with mammary gland activity to produce *de novo* FA, or, alternatively, C18:1 *cis*-9 in order to meet fluidity needs of milk, the trend of its scores during lactation indicates higher contents of de novo FA and lower of C18:1 *cis*-9, in early lactation than in middle and late lactation, respectively. This result is confirmed by the plot of the concentrations of *de novo* FA and C18:1 *cis*-9 during lactation (Figure 2) that exhibits a specular trend, and by their negative strong correlation ($r = –0.78$, $p < 0.0001$). A partial explanation could be found in the inhibitory effect of C18:1 *cis*-9 on the synthesis of *de novo* FA (Natali et al., 2007). The evolution of the *milk fat fluidity* factor during lactation was unexpected, as it has been reported that animals in early lactation are in negative energy balance and increase mobilization of adipose FA (Belyea and Adams, 1990). The consequent uptake of FA inhibits the lipogenic activity of the mammary gland, causing a reduction in milk concentration of *de novo* FA and an increase in C18:1 *cis*-9 (Mele et al., 2007). Our finding was also in disagreement with previous works on factor analysis of FA in cows (Conte et al., 2016; Mele et al., 2016). In these studies the factor associated with the *de novo* FA shows an opposite trend during lactation.

The scores of *desaturase* factor (Figure 1) were negative in early lactation, then increased reaching positive values in the middle lactation, and finally decreased to reach negative values in late lactation. This pattern seems suggest a higher activity of SCD of the mammary gland of animals in middle stage of lactation than that of animals in early and late stage of lactation. The expression and

activity of SCD are affected by several factors such as nutrition, genetics, environment, and physiological state of animals (Ntambi and Miyazaki, 2004). During lactation, several changes occur in the hormonal status of animals, involving regulation of enzyme expression and activities. For example, a positive correlation of SCD activity and blood insulin level was found. Studies on mice have demonstrated that insulin positively regulates the gene expression of SCD (Waters and Ntambi, 1994). Our finding was in agreement with the work of Mele et al. (2016), which observed increasing values for the scores of the factor associated with the SCD activity of the mammary gland of cows from early to mid-lactation and a decrease in late lactation.

The pattern of the scores of *BCFA* factor during lactation (Figure 3) showed negative values in the first part, and values closed to 0 (positives and negatives) in the middle and late lactation. This suggests that in the first part of lactation the content of BCFA in milk was lower than in middle and late lactation. Since BCFA are reported to be mainly produced by cellulolytic bacteria (Vlaeminck et al., 2006), the negative values of BCFA factor in early lactation may suggest a shift in rumen microbial ecology in response to dietary changes, with a reduction in the activity and growth of these bacteria. Variations in forage:concentrate ratio in the diet have been reported as one of the main factors affecting the relative abundance of different strains of ruminal microorganisms and, consequently, the concentration of BCFA (Vlaeminck et al., 2006).

4. Conclusions

Multivariate factor analysis could be a useful statistical tool to characterize the complex pattern of FA profile in buffalo's milk. The ten extracted factors were able to describe and synthesize the relationships between the milk FA, evidencing some common metabolic pathways. Some revealed independent association of FA of common origin with different latent factors that evidenced a different regulation of their production or excretion in milk. For example, the products of desaturase were independently associated with 3 latent factors, with different biological meanings: milk fat fluidity, ruminal biohydrogenation and desaturation. Essential FA *n*–3 PUFA and *n*–6 PUFA were

associated with two different factors, evidencing the relevance of the diet in the definition of milk FA composition, with important implications from a nutritional point of view.

The results of the mixed linear model highlighted the relationship between the latent factors and age, month of calving and stage of lactation, with the latter having the highest influence. The factor scores could be used as new phenotypes for characterising the FA profile of buffaloes and can be used as new traits for management and breeding purposes.

Commented [S1]: buffalo milk?

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Figure captions:

Figure 1. Lactation pattern of *milk fat fluidity* and *desaturase* factors.

Figure 2. Effect of stage of lactation (month) on content of C18:1 cis-9 and *de novo* FA in milk.

Figure 3. Lactation pattern of *BCFA* factor.

Figure 1.

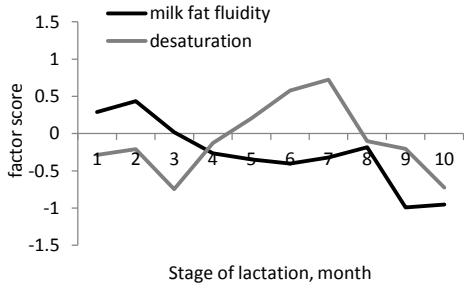

Figure 2.

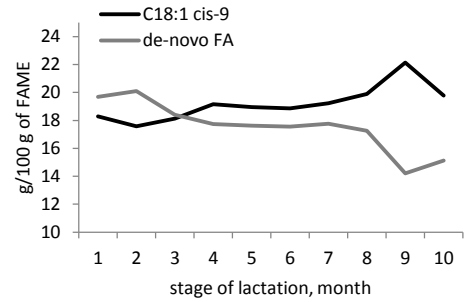

Figure 3.

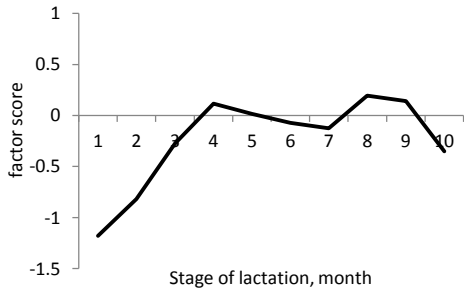

Table 1. Descriptive statistics for individual and classes of milk FA (*n* = 214)

	Mean	SD	CV(%)	[1]P1	[1]P5	[1]P95	[1]P99
Fatty acid g/100 g of FAME[2]							
C4:0	3.82	0.59	15.5	2.59	3.00	4.88	5.30
C6:0	1.77	0.33	18.5	1.01	1.18	2.27	2.44
C8:0	0.90	0.23	25.1	0.44	0.51	1.23	1.44
C10:0	1.92	0.56	29.3	0.85	1.05	2.88	3.33
C10:1 *cis*-9	0.08	0.03	38.9	0.03	0.04	0.14	0.16
C11:0	0.03	0.01	46.9	0.00	0.01	0.05	0.06
C12:0	2.55	0.68	26.8	1.33	1.48	3.79	4.18
iso C13:0	0.02	0.01	50.6	0.01	0.01	0.04	0.05
anteiso C13:0	0.04	0.01	21.9	0.02	0.02	0.05	0.06
C13:0	0.10	0.02	21.6	0.05	0.07	0.13	0.15
iso C14:0	0.20	0.04	21.7	0.11	0.13	0.27	0.29
C14:0	11.3	1.70	15.0	7.53	8.21	13.9	14.9
C14:1 *cis*-9	0.74	0.25	34.2	0.29	0.38	1.16	1.43
iso C15:0	0.34	0.06	17.5	0.23	0.25	0.44	0.49
anteiso C15:0	0.56	0.10	17.0	0.38	0.43	0.74	0.82
C15:0	1.20	0.16	13.3	0.83	0.94	1.43	1.59
iso C16:0	0.40	0.08	19.4	0.24	0.29	0.55	0.59
C16:0	35.2	3.21	9.13	28.3	30.0	40.4	42.3
C16:1 *trans*-9	0.05	0.02	32.7	0.00	0.03	0.09	0.11
C16:1 *cis*-7	0.20	0.04	21.1	0.13	0.15	0.26	0.34
C16:1 *cis*-9	2.04	0.68	33.4	1.01	1.17	3.44	4.02
iso C17:0	0.25	0.04	16.0	0.16	0.19	0.32	0.34
anteiso C17:0	0.38	0.07	17.8	0.26	0.29	0.52	0.56
C17:0	0.51	0.07	14.3	0.36	0.40	0.64	0.68
C17:1 *cis*-9	0.18	0.05	29.1	0.10	0.11	0.28	0.36
C18:0	10.4	2.35	22.6	6.09	7.11	14.7	16.2
C18:1 *trans*-4	0.10	0.05	50.1	0.00	0.04	0.18	0.27
C18:1 *trans*-6+7+8	0.14	0.07	50.5	0.04	0.04	0.27	0.35
C18:1 *trans*-9	0.16	0.07	46.5	0.05	0.06	0.28	0.37
C18:1 *trans*-10	0.25	0.10	42.1	0.08	0.10	0.42	0.54
C18:1 *trans*-11	0.91	0.32	35.6	0.39	0.53	1.67	1.99
C18:1 *cis*-9 + *trans*-13 + 14	18.7	3.11	16.6	13.2	14.2	24.3	26.4
C18:1 *cis*-10 + *trans*-15	0.29	0.18	62.4	0.06	0.09	0.68	0.80
C18:1 *cis*-11	0.56	0.11	18.9	0.34	0.43	0.74	0.89
C18:1 *cis*-12	0.24	0.08	32.6	0.10	0.13	0.39	0.46
C18:2 *trans*-9,*trans*-12	0.14	0.08	57.2	0.03	0.04	0.28	0.41
C18:1 *cis*-15	0.14	0.09	61.2	0.01	0.02	0.31	0.45
C18:2 *n*–6	1.51	0.35	23.5	0.81	1.03	2.18	2.56
C18:3 *n*–6	0.03	0.02	76.6	0.00	0.01	0.05	0.14
C18:3 *n*–3	0.23	0.08	32.5	0.12	0.15	0.38	0.57
CLA *cis*-9,*trans*-11	0.41	0.13	33.1	0.21	0.26	0.70	0.89
CLA *trans*-9,*cis*-11 + C20:0	0.19	0.05	27.1	0.10	0.12	0.28	0.33
CLA *trans*-11,*trans*-13	0.10	0.03	30.5	0.05	0.06	0.16	0.19
CLA *trans*-9,*trans*-11 + C20:1	0.04	0.02	41.9	0.00	0.02	0.06	0.08
C20:2 *n*–6	0.02	0.01	51.8	0.00	0.01	0.03	0.05
C20:3 *n*–9	0.05	0.02	31.6	0.02	0.02	0.08	0.09
C20:3 *n*–6	0.06	0.02	28.6	0.03	0.03	0.08	0.11
C20:4 *n*–6	0.11	0.03	29.2	0.05	0.06	0.16	0.21
C22:0	0.07	0.03	44.1	0.02	0.02	0.12	0.14
EPA (C20:5 *n*–3)	0.02	0.01	47.1	0.01	0.01	0.04	0.08

C24:0	0.03	0.02	53.5	0.00	0.01	0.06	0.08
DPA (C22:5 *n*–3)	0.04	0.01	37.2	0.01	0.02	0.06	0.08
SFA	72.0	3.97	5.51	61.1	64.7	77.6	79.4
MUFA	24.7	3.60	14.6	17.8	19.9	31.3	34.8
PUFA	3.09	0.53	17.2	1.83	2.38	4.07	4.26
BCFA	2.19	0.35	16.2	1.58	1.66	2.87	3.10
OBCFA	4.02	0.53	13.0	2.96	3.21	5.00	5.45
SCFA	8.54	1.46	17.1	5.35	6.11	10.8	11.5
MCFA	56.3	4.71	8.36	45.0	48.9	63.1	65.9
LCFA	35.1	5.65	16.1	24.7	27.0	44.7	48.6
PUFA *n*–3	0.31	0.09	30.1	0.15	0.21	0.46	0.75
PUFA *n*–6	1.74	0.38	22.1	0.95	1.27	2.48	2.87
n–6/*n*–3	5.95	1.46	24.5	1.97	3.79	8.45	9.46

[1]P1 = 1th percentile, P5 = 5th percentile, P95 = 95th percentile, P99 = 99th percentile.

[2]EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; BCFA: branched chain fatty acids; OBCFA: odd and branched chain fatty acids; SCFA: short chain fatty acids; MCFA: medium chain fatty acids; LCFA: long chain fatty acids; *n*–6/*n*–3: ratio between PUFA *n*–6 and PUFA *n*–3.

Table 2. Rotated factor pattern and variable communality (com)

	factors[2]										com
name[1]	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	
C8:0	**0.95**	-0.22	-0.03	-0.06	0.08	0.03	-0.03	-0.05	0.01	-0.03	0.97
C10:0	**0.95**	-0.20	-0.04	-0.03	0.07	0.06	-0.01	-0.04	0.05	0.04	0.96
C12:0	**0.94**	-0.21	-0.03	-0.03	0.14	0.05	-0.02	-0.01	0.06	0.07	0.95
C6:0	**0.89**	-0.23	-0.05	-0.18	-0.02	-0.10	-0.06	-0.08	-0.06	-0.17	0.94
C14:0	**0.85**	-0.35	-0.08	-0.13	0.07	-0.21	-0.13	-0.02	0.03	-0.03	0.94
C11:0	**0.69**	-0.14	-0.11	0.06	0.06	0.14	0.04	0.03	0.53	0.22	0.86
C10:1	**0.66**	-0.20	0.10	0.02	**0.61**	0.13	-0.01	0.03	0.11	0.12	0.91
C18:1 *cis*-9 + *trans*-13 + 14	**-0.63**	0.36	0.06	0.23	0.03	0.44	0.27	-0.05	-0.01	0.06	0.86
C18:1 *trans*-10	-0.21	**0.90**	-0.11	0.02	-0.14	0.10	0.03	-0.02	-0.06	0.10	0.92
C18:1 *trans*-9	-0.23	**0.89**	-0.08	0.05	-0.13	0.05	0.04	-0.05	-0.10	0.14	0.91
C18:1 *trans*-6+7+8	-0.25	**0.86**	-0.12	0.06	-0.11	-0.07	0.08	0.05	-0.13	0.18	0.91
C18:1 *trans*-11	-0.28	**0.77**	0.02	0.22	-0.23	0.13	-0.08	0.00	-0.09	-0.13	0.83
C18:1 *cis*-10	-0.24	**0.77**	-0.08	-0.24	-0.07	-0.07	0.02	0.09	-0.21	-0.19	0.81
C18:1 *cis*-12	-0.16	**0.73**	-0.06	-0.07	-0.16	0.25	-0.11	-0.01	0.11	-0.16	0.71
CLA *cis*-9,*trans*-11	-0.33	**0.65**	0.15	0.23	0.31	0.21	-0.10	0.07	0.01	-0.13	0.79
C18:1 *cis*-15	-0.19	0.56	-0.15	0.23	-0.05	0.34	-0.04	-0.13	0.18	-0.17	0.62
C18:2 *trans*-9,*trans*-12	-0.06	0.45	-0.23	0.16	0.02	-0.18	0.12	0.30	0.18	0.05	0.46
C16:1 *trans*-9	-0.21	0.44	0.13	0.11	0.14	0.23	0.34	0.04	0.15	-0.30	0.57
anteiso C15:0	-0.01	-0.03	**0.93**	0.08	0.17	0.05	0.11	0.06	0.12	-0.01	0.94
iso C15:0	-0.05	-0.10	**0.90**	0.00	0.10	0.13	-0.04	0.02	0.20	0.03	0.89
iso C16:0	0.02	-0.06	**0.89**	0.14	0.12	-0.07	0.11	0.08	-0.07	0.06	0.86
iso C14:0	0.05	-0.01	**0.85**	0.05	0.03	0.20	0.14	-0.08	0.11	-0.10	0.81
anteiso C17:0	-0.04	-0.11	**0.78**	0.30	0.19	-0.06	0.27	0.14	-0.13	0.13	0.88
anteiso C13:0	-0.02	0.02	**0.72**	-0.03	0.09	0.14	-0.20	-0.05	0.03	0.01	0.59
iso C17:0	-0.21	-0.20	**0.71**	0.30	0.03	0.01	0.19	0.03	-0.14	0.26	0.80
C22:0	-0.06	0.08	0.08	**0.92**	-0.07	0.10	-0.01	0.12	0.10	-0.07	0.90
C24:0	0.00	-0.01	0.28	**0.84**	0.05	0.21	-0.01	-0.05	-0.03	-0.03	0.84
CLA *trans*-9,*cis*-11 + C20:0	-0.26	0.15	-0.01	**0.79**	-0.30	0.11	-0.07	0.18	0.04	-0.04	0.86
C20:3 *n*–9	0.08	0.01	0.18	**0.78**	0.03	0.04	0.20	0.00	-0.08	0.20	0.74
CLA *trans*-11,13	-0.31	0.26	0.23	**0.58**	0.37	0.19	0.04	0.31	0.01	-0.03	0.82
CLA *trans*-9,11 + C20:1 *n*–9	-0.30	0.17	-0.19	0.40	-0.05	0.23	0.04	0.19	-0.25	0.00	0.47
C16:1 *cis*-9	-0.11	-0.15	0.28	-0.10	**0.88**	-0.10	-0.01	0.10	0.03	0.04	0.93
C14:1 *cis*-9	0.31	-0.16	0.26	-0.08	**0.86**	0.04	-0.10	-0.01	0.05	0.02	0.95
iso C13:0	0.56	-0.12	0.10	0.08	**0.62**	0.25	-0.03	-0.04	0.10	0.11	0.82
C17:1 *cis*-9	-0.15	-0.06	0.43	0.27	0.55	-0.10	0.48	0.20	0.01	0.12	0.88
C18:0	-0.37	0.27	-0.02	0.27	**-0.67**	0.32	-0.02	-0.14	-0.05	-0.08	0.86
C20:3 *n*–6	0.04	0.18	0.25	0.29	0.04	**0.61**	0.16	-0.02	0.08	0.08	0.60
C20:2 *n*–6	-0.06	0.13	0.03	0.09	-0.09	**0.53**	-0.36	0.12	-0.05	0.17	0.50
C20:4 *n*–6	0.26	-0.05	0.23	0.28	0.11	0.44	0.30	0.20	-0.05	0.32	0.64
C16:0	-0.07	-0.45	-0.20	-0.36	0.16	**-0.61**	-0.22	0.11	-0.01	0.04	0.83
C16:1 *cis*-7	-0.13	0.02	0.23	-0.03	-0.01	0.08	**0.87**	0.02	-0.03	-0.02	0.83
C17:0	-0.15	-0.05	0.39	0.52	-0.22	-0.17	0.55	0.19	0.11	0.04	0.86
C18:1 *cis*-11	-0.07	0.26	-0.12	0.44	-0.29	0.19	0.51	0.10	0.09	0.18	0.72
EPA	-0.01	0.09	-0.01	0.27	0.08	0.04	-0.08	**0.78**	-0.08	-0.24	0.77
C18:3 *n*–3	-0.10	0.01	0.10	-0.10	0.09	-0.10	0.03	**0.75**	0.16	0.26	0.71
DPA	0.00	-0.12	0.11	0.34	0.01	0.20	0.29	**0.65**	-0.13	0.18	0.74
C13:0	0.58	-0.13	0.30	-0.01	0.04	0.12	0.03	-0.07	**0.65**	0.09	0.89
C15:0	0.21	-0.10	0.57	0.11	0.17	-0.20	0.19	0.11	**0.62**	0.13	0.91
C18:1 *trans*-4	-0.03	0.39	-0.08	0.15	-0.23	-0.06	0.21	-0.04	-0.39	0.33	0.54
C18:3 *n*–6	0.06	-0.08	0.13	-0.02	0.11	0.09	-0.02	0.06	0.05	**0.62**	0.44
C4:0	0.34	-0.17	-0.05	-0.39	-0.13	-0.31	-0.05	-0.12	-0.23	-0.49	0.72
Eigenvalue	7.57	6.60	6.39	5.19	3.97	2.57	2.54	2.18	1.79	1.66	
Var. explained (%)	14.85	12.94	12.53	10.17	7.78	5.05	4.97	4.27	3.51	3.25	

[1] EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid;

[2] Factors, F1: milk fat fluidity; F2: biohydrogenation; F3: branched chain fatty acids (BCFA); F4: long chain fatty acids (LCFA); F5: desaturase; F6: PUFA omega-6 (*n*–6); F7: C16:1 *cis*-7; F8: PUFA omega-3 (*n*–3); F9: odd chain fatty acids (OCFA); F10: γ-linolenic acid.

Table 3. Analysis of variance (*F* and *P* values) of the effect of age (DF, 5), month of calving (DF, 11) and DIM (DF, 9) on the 10 extracted factors

Item[1]		age		calving month		DIM	
		F	*P-value*	F	*P-value*	F	*P-value*
F1	*milk fat fluidity*	0.89	0.489	2.53	**0.006**	2.15	**0.028**
F2	*biohydrogenation*	0.30	0.909	0.57	0.853	1.21	0.291
F3	*BCFA*	0.53	0.753	1.46	0.151	2.48	**0.011**
F4	*LCFA*	1.40	0.229	1.20	0.291	2.63	**0.007**
F5	*desaturase*	1.31	0.263	2.24	**0.015**	2.27	**0.020**
F6	*n–6*	0.60	0.699	1.26	0.255	0.34	0.962
F7	*C16:1 cis-7*	1.58	0.170	1.15	0.324	2.43	**0.013**
F8	*n–3*	0.41	0.842	1.41	0.173	0.59	0.804
F9	*OCFA*	2.56	**0.029**	1.15	0.323	0.71	0.697
F10	*γ-linolenic acid*	1.36	0.243	1.07	0.386	1.24	0.277

[1]Item: F1: milk fat fluidity; F2: biohydrogenation; F3: branched chain fatty acids (BCFA); F4: long chain fatty acids (LCFA); F5: desaturase; F6: PUFA omega-6 (*n*–6); F7: C16:1 *cis*-7; F8: PUFA omega-3 (*n*–3); F9: odd chain fatty acids (OCFA); F10: γ-linolenic acid

Table 4. Effect of month of calving on milk fat fluidity and desaturase latent factors

factors	month of calving*											
	1	2	3	4	5	6	7	8	9	10	11	12
milk fat fluidity	0.40^{a}	$–0.47^{ab}$	$–0.28^{ab}$	$–0.08^{ab}$	$–0.20^{ab}$	$–0.09^{ab}$	$–0.09^{ab}$	0.35^{a}	$–0.25^{ab}$	$–0.35^{ab}$	$–1.11^{b}$	$–1.09^{b}$
desaturase	0.06^{ab}	0.45^{ab}	0.81^{a}	0.39^{ab}	$–0.69^{b}$	$–0.15^{ab}$	$–0.30^{ab}$	$–0.09^{ab}$	$–0.12^{ab}$	$–0.65^{b}$	$–0.28^{ab}$	$–0.51^{ab}$

[a–b] Values within the same row without a common superscript differ ($p < 0.05$)

*Month of calving from 1to12: from January to December