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

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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.

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 t/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-Gotet et 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.,

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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 $\pm$ 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 $\times$ 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 $\geq$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 Ménard 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; Ménard 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