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Investigating mutual relationship among milk fatty acids by multivariate factor analysis in dairy cows

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ABSTRACT

The interpretation of mutual relationship among milk fatty acids (FA) is not easy due to the high number of FA contained in milk fat and to the high degree of correlation among them. Multivariate analysis includes different statistical approaches that could help explaining complex pattern of variables. In this study, Multivariate Factor Analysis (MFA) was used to decompose the correlation matrix of 47 FA and milk production traits (milk yield and protein and fat content) measured in 300 Italian Holstein Friesian cows reared in the North of Italy in 23 commercial dairy farms, representative of the intensive dairy system. MFA was able to extract seven latent factors with specific biologic meaning: secretion of Long Chain FA (K_{LCFA}), mammary FA de novo synthesis (K_m), rumen biohydrogenation (K_{bh}), synthesis of odd chain FA (K_o), synthesis of branched chain FA (K_b), mammary desaturation activity (K_d), milk yield (K_{my}). According to the pattern of communalities of the factor analysis, C18:3c9c12c15 was the only FA, along with C18:2t11c15, to be uncorrelated with the other variables and it seemed to be excluded by the metabolic pattern described by the seven factors. The desaturation products of the SCD enzyme were independently associated to three latent factors, suggesting new insights in the regulation of SCD activity. Factors were considered as new quantitative phenotypes related to prominent features of milk FA profile. With the aim of evaluating the feeding regimen and animal effects, latent factors were analysed with a mixed model, which considered the fixed effect of lactation stage, parity, some feeding regimen characteristics and the random effect of bull. Lactation stage significantly affected K_m and K_{my} factors. In perspective, the seven factors extracted by applying MFA analysis to milk FA composition could be considered as new and more informative traits to test the effect of endogenous and exogenous variation factors.

1. Introduction

The ruminant milk fatty acid (FA) composition depends on intrinsic (species, breed, genotype and lactation stage) and extrinsic (feeding and management) factors (Chilliard et al., 2007). Milk FA derive for nearly 60% from de novo synthesis in the mammary gland from acetate and butyrate and for the remaining part from preformed FA introduced with the diet (eventually affected by rumen biohydrogenation processes) or derived from fat mobilization (Palmquist et al., 1993).

As regard intrinsic factors, the effects of individual variations (breed, gene polymorphisms and animal physiologic status) on milk FA composition have been extensively evaluated in several dairy cattle populations (Kay et al., 2005; Mele et al., 2007; Soyeurt et al., 2007; Stoop et al., 2008; Mele et al., 2009). These sources of variation explain a significant level of total variance, but smaller than that explained by extrinsic factors related, especially to feeding regimen.

Diet may significantly affect milk FA amounts by: (i) providing higher amount of dietary FA; (ii) modulating the amount of acetate and butyrate for mammary de novo synthesis; (iii) influencing the rumen biohydrogenation of dietary FA. Moreover, some biohydrogenation intermediates are able to interfere with FA metabolism in the mammary gland (Buccioni et al., 2012; Shingfield et al., 2013). The amount and type of fat added to the diet, as well as of forage adopted are the main dietary factors able to affect milk FA composition (Shingfield et al., 2013).

In the last 20 years, FA analytical techniques (i.e. gas-chromatography) largely improved, making easier to detect routinely a large number of milk FA. So, it is now possible to obtain a detailed milk FA profile, composed by more than 45 individual FA, including several positional and geometric isomers of C16:1, C18:1, C18:2 and C18:3 groups (Del Monte et al., 2012). Increasing the

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number of FA detected made the interpretation of the FA pattern more complex. Statistical approaches based on data-reduction methods, such as principal component analysis (PCA) and multivariate factor analysis (MFA), may help simplifying the overall pattern. These methods are able to synthesized complex phenotypes to linear combinations of original data whose weights are objectively derived from the correlation matrix of the original variables (Macciotta et al., 2004). Particularly, MFA examines relationship between the original variables and extract few unobservable latent variables, called common factors, which are able to explain particular characteristic of the original variables. Therefore, these factors can be considered as new phenotypes and used for further analyses.

Another interesting feature of common factors is that they are uncorrelated or weakly correlated. Thus, it is possible to investigate the effects of a specific component of the variability of the complex system of milk FA composition using few uncorrelated variables.

Multivariate analysis has been applied to milk FA composition in dairy cow with different purposes: to evaluate the origin of heptadecenoic and conjugated linoleic acids in milk (Fievez et al., 2003), to study the milk FA pattern during the milk fat depression (Kadegowda et al., 2008), to discriminate the area of origin of bulk milk (Gaspardo et al., 2010). Several studies also adopted MFA on milk components in order to achieve different objectives: to obtain an overview of the relationship between milk minerals (Rodrìguez Rodrìguez et al., 1999); to study the pattern of milk composition in goat milk (Todaro et al., 2005); to modelling shapes of individual lactation curves in dairy cattle (Macciotta et al., 2004); to evaluate the physiological regulation of milk composition (Bobe et al., 1999); to study the milk coagulation properties in Brown Swiss cows (Macciotta et al., 2012).

To our knowledge, this is the first time that MFA was applied to detailed individual milk FA composition in order to investigate mutual metabolic relationships between milk FA. Previous studies adopted only PCA (Fievez et al., 2003; Kadegowda et al., 2008) or included in the elaboration a limited number of FA, considering the most abundant FA or class of FA (Bobe et al., 1999; Konuspayeva et al., 2009).

The aim of this study was to investigate the structure of mutual relationships among FA of individual milk samples obtained from cows reared in commercial dairy farms that were representative of the feeding conditions adopted in the intensive dairy system by the application of MFA. Since MFA was applied to FA composition of milk samples obtained from commercial dairy farms, where the dietary treatments were not a priori defined and the differences among diets were smaller than that usually adopted in feeding experiments, an other objective of the study was to evaluate the ability of MFA to explain the milk FA variability as affected by feeding regimen and some endogenous factors (parity and phase of lactation).

2. Material and methods

2.1. Animals, feeding regimen, diet composition and samples collection

The study involved 300 Italian Holstein cows reared in 23 commercial dairy farms (13 ± 4 cows per farm). These farms adopted feeding regimens representative of the intensive dairy systems of the North of Italy, the most representative Italian area for the production milk and cheese (i.e. Grana Padano DPO). Milk samples were collected one time per cows.

Days in milking (DIM) ranged from 50 to 300 (average 224 ± 88) and individual milk yield ranged from 11 to 54 kg/d (average 28.5 ± 10.2 kg/d). Using a specific questionnaire, data related to feeding regimen and to diet composition were recorded for each farm involved in the study, taking into consideration the last 20 days before the date of milk sampling. Data about amount of corn silage in the diet, forage to concentrate ratio and use of dietary lipid supplements fell in the range reported by Guerci et al. (2013) and Borreani et al. (2013), who specifically described the characteristics of feeding regimen in intensive dairy farms located in the same geographical area. Hence, data of milk FA composition were also representative of the ordinary management of dairy cows in the considered region. Feeding regimen was based on a total mixed ration (TMR) directly formulated by farmers or their technicians on the basis of their experience and the productive goals of the farm. Cows never graze and no fresh forage was included in the diet. TMR contained only preserved forages and concentrates as reported in Table 1: four farms used alfalfa hay, twelve farms grass hay, and seven farms a mix of alfalfa and grass hay; corn silage was used in all farms and twelve farms used grass silage too; only four farms did not use fat supplementation or high fat grain in the diet. The total amount of concentrate in the ration ranged from 32–60% of DM offered. The average amount of DM offered to each animal was 23 ± 3 kg per day, ranging from 19.1 to 28.3 kg.

2.2. Extraction and analysis of milk fatty acids

Methyl esters of FA were prepared by a direct extraction and alkali catalyzed transmethylation procedure, as follows: milk sample was centrifuged at $5000 \times g$ for 30 min at 4 °C to facilitate the surfacing of the fat. Fat was collected and transferred in a new tube, maintained at 40 °C for 20 min and centrifuged at $13{,}000 \times g$ for 20 min Twenty mg of the upper layer were collected in a fresh amber vial and eluted with 1 mL of hexane and 0.5 mL of 0.5 N methanolic solution of sodium hydroxide. After an incubation of 5 min at room temperature, 0.25 mg of $NaHSO_3 \cdot H_2O$ were also added. Subsequently, the samples were centrifuged at $13{,}000 \times g$ for 5 min at 4 °C and the surfactant layer was collected in a vial for gas-chromatographic (GC) analysis. The FA composition was determined by GC using a GC2010 Shimadzu gas chromatograph (Shimadzu, Columbia, MD, USA) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil88 Varian, Middelburg, the Netherlands; 100 m, 0.25 mm i.d.; film thickness 0.20 μm). Hydrogen was used as the carrier gas at a flow of 1 mL/min. Split/splitless injector was used with a split ratio of 1:80. An aliquot of the sample was injected under the following GC conditions: the oven temperature started at 60 °C and held at that level for 1 min; it was then increased to 173 °C at a rate of 2 °C/min, and held at that level for 30 min, before being once again increased to 185 °C at 1 °C/min and held for 5 min, and then to 220 °C at a rate of 3 °C/min, and held for 19 min The injector temperature was set at 270 °C and the detector temperature was set at 300 °C. Individual FA methyl esters were identified by comparison with a standard mixture of 52 Component FAME Mix (Nu-Chek Prep Inc., Elysian, MN). The identification of isomers of 18:1 was based on commercial standard mixtures (Supelco, Bellefonte PA) and published isomeric profiles (Kramer et al., 2008). A reference standard butter (BCR 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for Short Chain FA (SCFA, 4:0 to 10:0), according to Mele et al. (2009). Milk FA composition was expressed as grams per 100 *g* of FA.

2.3. Statistical analysis

Statistical analysis was performed by JMP software (SAS Institute Inc., Cary, NC).

MFA is multivariate dimension reduction techniques able to synthesize information contained in a set of n observed variables (y1, …, yn) by seeking a new set of p ($p < n$) variables (X1,…, Xp), termed common latent factors.

Factor analysis considers the variance of each original variable as a combination of common and unique components, named