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Principal Component and Multivariate Analysis of Milk Long-Chain Fatty Acid Composition During Diet-Induced Milk Fat Depression

A. K. G. Kadegowda, L. S. Piperova, and R. A. Erdman[1]
Animal and Avian Sciences Department, University of Maryland, College Park 20742

ABSTRACT

The objective of this study was to assess the relationship between individual milk fatty acids (FA) and diet-induced milk fat depression (MFD) using principal component analysis (PCA) and multivariate analysis (MA). Cow treatment observations (n = 63) from 3 published feeding experiments with lactating dairy cows were used in the analyses. In the PCA, principal component loading plots 1 (PC1) and 2 (PC2) described 55.9% of the total variation in milk FA and fat concentrations. Saturated FA (14:0, 16:0, and 17:0) and milk fat percentage showed negative loading for PC1. *Trans*-18:1 isomers (*trans*-6+7+8 to *trans*-15), *trans*-7, *cis*-9 conjugated linoleic acid (CLA), and *trans*-10, *cis*-12 CLA showed positive (opposite) loading, suggesting a negative relationship between these isomers and milk fat percentage. *Cis*-11, *trans*-13 CLA and *cis*-9, *trans*-11 CLA were associated with the PC2 axes (neutral), indicating that they were not associated with MFD. Multivariate analysis with milk fat percentage as the dependent variable and individual PC1 positive loading variables showed a breakpoint relationship for *trans*-6+7+8-, *trans*-9-, *trans*-10-, and *trans*-13+14-18:1 and a linear relationship for *trans*-11-, *trans*-12-, *trans*-15-18:1, *trans*-10, *cis*-12 CLA, and *trans*-7, *cis*-9 CLA. Subsequent MA was conducted on 41 treatment means from 12 independent experiments from the literature, in which concentrations of *trans*-6+7+8-, *trans*-9-, *trans*-10-, and *trans*-11-18:1, and *cis*-9 *trans*;-11, and *trans*-10, *cis*-12 CLA were reported. Significant negative effects of *trans*-9-18:1, *trans*-10-18:1, and *trans*-10, *cis*-12 CLA on milk fat percentage were observed. In this study, the PCA and MA showed that among *trans*-18:1 isomers, *trans*-10-18:1 was the most negatively correlated to milk fat percentage. However, the threshold concentration related to maximum MFD indicated that the relative potency was greatest for *trans*-6+7+8- and lowest for *trans*-10-18:1. These results suggested that *trans*-6+7+8-18:1 might be more important than *trans*-10-18:1 in MFD. Principal component analysis also showed that *trans*-10, *cis*-12 and *trans*-7, *cis*-9 CLA were the isomers most negatively correlated to milk fat percentage, implying a possible role of *trans*-7, *cis*-9 CLA in MFD. Additional experiments are needed to establish whether *trans*-7-18:1 is involved in MFD or that its effects are mediated via the endogenously synthesized *trans*-7, *cis*-9 CLA.
Key words: milk fat depression, fatty acid, principal component analysis, multivariate analysis

INTRODUCTION

It has been demonstrated that conjugated linoleic acid (**CLA**) and *trans*-18:1 fatty acids (**FA**) arising from incomplete rumen biohydrogenation of dietary polyunsaturated fatty acids (**PUFA**) can markedly alter milk fat synthesis. Numerous studies have shown that concentrations of *trans*-18:1 FA or CLA can be increased in milk via dietary means (Griinari et al., 1998; Piperova et al., 2000; Peterson et al., 2003) or abomasal infusion (Gaynor et al., 1994; Romo et al., 1996; Chouinard et al., 1999) and can cause reduction of milk fat concentration. Examination of the isomer profile of *trans*-18:1 FA (Griinari et al., 1998) and CLA (Griinari and Bauman., 1999) indicated that increases in ruminally derived *trans*-10-containing 18:1 and 18:2 isomers in milk were more closely associated with milk fat depression (**MFD**) than the general increase in total *trans*-18:1 or CLA. We were able to demonstrate that abomasal infusion of partially hydrogenated vegetable oil (Gaynor et al., 1994; Romo et al., 1996) or diets supplemented with Ca salts of *trans*-18:1 containing isomers from *trans*-(**t**)6 to t16 (Piperova et al., 2004) can cause MFD. However, when individual *trans*-18:1 isomers, including t9- (Rindsig and Schultz, 1974), t11-, and t12- (Griinari et al., 2000) were postruminally infused, milk fat was not affected. Although high concentrations of t10-18:1 are typically observed in the milk fat of lactating cows fed MFD diets (Piperova et al., 2000; Peterson et al., 2003; Loor et al., 2004), abomasal infusion of 40 g/d of t10-18:1 was not effective in reducing milk fat percentage in lactating cows (Lock et al.,

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[1]Corresponding author: erdman@umd.edu

Table 1. Details of the experimental data used in the principal components analysis

Reference	Observations/ treatment	Total observations	Treatments
Piperova et al., 2000	11	22	Control diet (forage:concentrate 60:40); MFD[1] diet (25% forage and 70% concentrate with 5% soybean oil).
Piperova et al., 2002	4	16	High forage (60% forage) with or without buffer addition; Low forage (25% forage) with or without buffer addition.
Piperova et al., 2004	5	25	Control; 100, 200, or 400 g of Ca-tFA[2] supplement; 100 g of Ca-conjugated linoleic acid supplement.

[1]Milk fat depression.
[2]Calcium salts of *trans*-18:1 fatty acids.

2007). Baumgard et al. (2000) provided convincing evidence that abomasal infusion of t10 *cis*-(**c**)12 CLA inhibits milk fat synthesis. Nevertheless, in an earlier study, Chouinard et al. (1999) observed MFD in lactating cows abomasally infused with a CLA mixture lacking t10c12 CLA.

Feeding experiments have shown that the concentration of t10c12 CLA in milk does not always account for the degree of MFD (Peterson et al., 2003) and that reduction in milk fat concentration can occur without increases in t10c12 CLA in milk (Piperova et al., 2004; Loor et al., 2005a). The t8c10 CLA and c11t13 CLA isomers usually present in lower concentrations in commercial CLA mixtures were examined by Perfield et al. (2004); they found no effect of these isomers on milk fat synthesis. These collective results suggest that there must be other biohydrogenation intermediates besides t10c12 CLA involved with MFD.

Multivariate analysis (**MA**) can be applied to visualize the multidimensionality of the data to identify underlying variables that may contribute to dietary MFD. Principal component analysis (**PCA**) is a multivariate technique that reduces the dimensionality of data by transforming a number of related variables into a set of uncorrelated variables, while retaining as much variation as possible. The transformed new variables, referred to as principal components (**PC**), are the linear combinations of the original variables. The first PC (PC1) accounts for the maximum variability, whereas the remaining PC (PC2, PC3, … PCn, n = number of variables) account for the remaining variability in the data. Each PC is independent and orthogonal to the other. Generally, only the first few PC describe the majority of total variation in the data as indicated by the Eigen values (Kent and Coker, 1992; Jolliffe, 2002). As a means to potentially identify individual FA effects, the objective of this study was to assess the relationship between milk FA and diet-induced MFD using PCA and MA.

MATERIALS AND METHODS

Experimental Design and Diets

Individual cow-within-treatment observations (n = 63) from 3 published feeding experiments conducted at the University of Maryland (Piperova et al., 2000, 2002, 2004) with lactating dairy cows were used in this analysis. The studies comprised diets with different forage-to-concentrate ratios (with or without vegetable oil or buffer addition) and diets supplemented with Ca salts of *trans*-18:1 FA or CLA. Diets were formulated to meet NRC (2001) requirements for milk production at 40 kg/d and 3.5% fat. Details of the experiments used in the study are presented in Table 1. In each experiment, cows were housed in individual stalls and fed individually. Forage and concentrate DM was measured weekly, and the TMR was adjusted accordingly to maintain constant forage-to-concentrate ratio on a DM basis during the experiment. Milk production was recorded at each milking. At the end of each experimental period, milk samples from 6 consecutive milkings were collected and composited for FA analysis.

Analytical Methods

The FA composition of milk samples and Ca-*trans* FA and Ca-CLA supplements was determined using FA methyl esters (**FAME**) prepared by mild transesterification with 0.04 *M* H_2SO_4 in methanol, at room temperature, using GLC conditions previously described (Piperova et al., 2000). *Trans*-18:1 concentration in milk and isomer distribution were determined by a combination of preparative Ag^+-thin layer chromatography and GLC analysis (Piperova et al., 2000). Argentation-HPLC was used to determine CLA isomer distribution patterns (Piperova et al., 2000).

Statistical Analysis

Relationships between milk FA were evaluated from PCA loading plots, based on the correlation matrix,