J. Dairy Sci. 101:1–14
https://doi.org/10.3168/jds.2017-13941


# A canonical discriminant analysis to study the association between milk fatty acids of ruminal origin and milk fat depression in dairy cows

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## ABSTRACT

Although milk fat depression (MFD) has been observed and described since the beginning of the last century, all the molecular and biochemical mechanisms involved are still not completely understood. Some fatty acids (FA) originating during rumen biohydrogenation have been proposed as causative elements of MFD. However, contradictory results were obtained when studying the effect of single FA on MFD. An alternative could be the simultaneous evaluation of the effect of many FA using a multivariate approach. The aim of this study was to evaluate the relationship between individual milk FA of ruminal origin and MFD using canonical discriminant analysis, a multivariate technique able to distinguish 2 or more groups on the basis of a pool of variables. In a commercial dairy herd, a diet containing 26% starch on a DM basis induced an unintentional MFD syndrome in 14 cows out of 40. Milk yielded by these 14 animals showed a fat content lower than 50% of the ordinary value, whereas milk production and protein content were normal. The remaining 26 cows secreted typical milk fat content and therefore were considered the control group, even though they ate the same diet. The stepwise discriminant analysis selected 14 milk FA of ruminal origin most able to distinguish the 2 groups. This restricted pool of FA was used, as variables, in a run of the canonical discriminant analysis that was able to significantly discriminate between the 2 groups. Out of the 14 FA, 5 conjugated linoleic acid isomers (C18:2 *trans*-10,*trans*-12, C18:2 *trans*-8,*trans*-10, C18:2 *trans*-11,*cis*-13, C18:2 *cis*-9,*cis*-11, C18:2 *cis*-10,*cis*-12) and C15:0 *iso* were more related to the control group, whereas C18:2 *trans*-10,*cis*-12, C16:1 *trans*-6–7, C16:1 *trans*-9, C18:1 *trans*-6–8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *cis*-11, and C18:3n-3 were positively associated with the MFD group, allowing a complete discrimination. On the basis of these results, we can conclude that (1) the shift of ruminal biohydrogenation from C18:1 *trans*-11 to C18:1 *trans*-10 seemed to be strongly associated with MFD; (2) at the same time, other C18:1 *trans* isomers showed a similar association; (3) on the contrary, conjugated linoleic acid isomers other than C18:2 *trans*-10,*cis*-12 seemed to be associated with a normal fat secretion. Results confirmed that MFD is the consequence of a combined effect of the outflow of many ruminal FA, which collectively affect mammary fat synthesis. Because the animals of the 2 groups were fed the same diet, these results suggested that factors other than diet are involved in the MFD syndrome. Feeding behavior (i.e., ability to select dietary ingredients in a total mixed ration), rumen environment and the composition of ruminal bacteria are additional factors able to modify the products of rumen biohydrogenation. Results of the present work confirmed that the multivariate approach can be a useful tool to evaluate a metabolic pathway that involves several parameters, providing interesting suggestions about the role of some FA involved in MFD. However, results about the MFD syndrome obtained in the present research require a deep molecular investigation to be confirmed.
**Key words:** milk fat depression, cow, biohydrogenation, discriminant analysis

## INTRODUCTION

Milk fat depression (**MFD**) syndrome is a phenomenon characterized by a reduction of milk fat level with no change in the secretion of milk protein and lactose (Shingfield and Griinari, 2007; Harvatine et al., 2009; Bauman et al., 2011).

Reduction in milk fat synthesis is generally related to the diet composition: low forage to concentrate ratio, high starch diets, and high vegetable or marine oil supplementation (Loor et al., 2005; Alizadeh et al., 2012; Kargar et al., 2012).

In the last 20 yr, several works proposed the biohydrogenation theory as the most comprehensive one to explain MFD. According to this theory, changes in rumen lipid metabolism may result in the formation

Received October 4, 2017.
Accepted February 26, 2018.
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of specific biohydrogenation intermediates that directly inhibit milk fat synthesis. Numerous works demonstrated that some specific dietary factors may influence ruminal pH and the microbial population, altering the pathways of rumen biohydrogenation of dietary fatty acids (**FA**). These factors included type of grain (Mohammed et al., 2010), amount and type of fat supplementation (Alizadeh et al., 2012; Kargar et al., 2012), forage to concentrate ratio, and forage type (Loor et al., 2005; Kargar et al., 2012). The molecular mechanisms involved in the mammary gland metabolism are still not completely known, but several works in dairy cows demonstrated a downregulation of the expression of key genes involved in milk fat synthesis (Angulo et al., 2012; Hussein et al., 2013; Bionaz et al., 2015).

Numerous studies investigated the role of ruminal FA on MFD, with conflicting results. The C18:2 *trans*-10,*cis*-12, a CLA isomer formed during the isomerization of C18:2n-6 in the rumen (Wallace et al., 2007), is the only biohydrogenation intermediate with an unequivocally demonstrated inhibitory effect on milk fat synthesis in dairy cows (Baumgard et al., 2002). Nevertheless, diet-induced MFD is a natural phenomenon able, under specific dietary compositions, to modify the pathways of rumen biohydrogenation producing FA intermediates that are potent inhibitors of milk fat synthesis (Bauman et al., 2011). The response of mammary gland to the effect of C18:2 *trans*-10,*cis*-12 is variable. The reaction is rapid to abomasal infusion (milk fat yield decreased after 14–60 h; Harvatine and Bauman, 2011). Also, a rapid recovery of normal milk fat was observed after the cessation of abomasal infusion of C18:2 *trans*-10,*cis*-12 (Baumgard et al., 2000). Feeding rumen-protected C18:2 *trans*-10,*cis*-12 induced a maximal decrease of milk fat yield within 1 wk of dietary intervention (Medeiros et al., 2010). On the contrary, the induction of MFD through dietary modification requires 11 to 19 d for complete induction (Shingfield et al., 2006) because changes in the rumen environment and a shift in the microbial population must occur to produce sufficient quantities of milk-fat-depressing intermediates to affect mammary lipid synthesis (Rico and Harvatine, 2013).

However, rumen outflow of C18:2 *trans*-10,*cis*-12 does not completely explain the decrease in milk fat synthesis in all cases of MFD, especially in small ruminants, in which a more variable response to lipid supplementation was observed (Toral et al., 2015). Moreover, increases in milk fat C18:2 *trans*-10,*cis*-12 concentrations on diets causing MFD are often lower than would be expected based on the observed enrichment in milk fat to postruminal C18:2 *trans*-10,*cis*-12 infusion, suggesting other biohydrogenation intermediates or other mechanisms may also be involved (Toral et al., 2015). According to these findings, several authors suggested that additional biohydrogenation intermediates other than C18:2 *trans*-10,*cis*-12 should be involved in the MFD syndrome (Shingfield and Griinari, 2007; Bichi et al., 2013; Toral et al., 2015). Other CLA isomers have been also suggested as responsible for MFD, such as C18:2 *trans*-9,*cis*-11 or C18:2 *cis*-11,*trans*-13 (Roy et al., 2006; Shingfield et al., 2006). Among C18:1 *trans* isomers, C18:1 *trans*-10, originating from C18:2 *trans*-10,*cis*-12, has been proposed as a repressor of mammary lipogenesis (McKain et al., 2010). In fact, Lock et al. (2007) revealed that abomasal infusion of C18:1 *trans*-10 has no effect on milk fat secretion. Overall, the increase of ruminal outflow of C18:1 *trans*-10 cannot completely explain the MFD in lactating cows (Shingfield and Griinari, 2007).

The effect of a single FA on MFD is usually evaluated by abomasal infusion. This approach allows obtaining information on the direct role of the single FA on MFD, but it does not provide any information about the pattern of rumen biohydrogenation related to the origin of a specific FA. Numerous C18:3, C18:2, and C18:1 isomers are usually produced in the rumen in the case of diet-induced MFD, and it is not possible to fully explain the observed decrease in milk fat yield by considering only one isomer (Ventto et al., 2017). So, the evaluation of MFD is very difficult because numerous variables affect the lipid metabolism.

The effect of biohydrogenation on MFD could be better evaluated by using a multivariate approach. Kadegowda et al. (2008) used principal component analysis to analyze the variability of FA composition observed in milk of cows affected by MFD. In the present work, the multivariate discriminant analysis was used to analyze the correlation structure of involved ruminal FA variables. The extracted information were used either to maximize distance between different groups or to better understand the phenomenon under study.

The main objective of this study was to develop a multivariate approach to discriminate cows affected by the MFD syndrome versus cows not affected, to identify specific milk FA patterns as potential markers of MFD.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

In a commercial dairy herd located in Tuscany (Italy), 14 cows out of 40 were classified by MFD status (MFD group), since they produced milk with a 50% reduction of fat content (Griinari et al., 1998) and normal level of yield and protein, as revealed during routine milk composition analysis. The remaining 26