Relationships Among Early Lactation Milk Fat Depression, Cattle Productivity and Fatty Acid Composition on Intensive Dairy Farms in Northern Italy

Luciano Comino, Federico Righi, Mauro Coppa, Afro Quarantelli, Ernesto Tabacco & Giorgio Borreani

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Introduction

Several researchers focused on identifying indicators in milk composition to support feeding, fertility and health management of the herd (Mottram et al., 2002; Rasmussen, 2004). Alteration in milk constituents and visual appearance of milk provide valuable information on the health and reproductive status of the cow (Brandt et al., 2010). Despite the relevant progresses made in dairy farming, the increased productivity has been associated with a growing percentage of anomalies in milk composition, such as milk fat depression (MFD). Parameters like milk fat and protein contents can reflect metabolic disorders and nutritional deficiencies or imbalances (Brandt et al., 2010) and can be evaluated to detect the occurrence of metabolic changes in the herd. Various attempts have been made to establish thresholds or to find a correct approach to interpret these values. Milk fat to protein ratio (FPR) has been proposed as a more sensitive indicator of metabolic status of dairy cattle than milk fat or protein contents considered separately (Brandt et al., 2010), and it has been reported to be negatively correlated to energy balance by Grieve et al. (1986). However, its value is affected by protein level, which is related to a physiologic process independent from fat synthesis and can be valuable mainly in supporting a diagnosis of subclinical ketosis (Oetzel, 2007, 2012). Moreover, Meinert et al. (1989), and Vos and Groen (1998) estimated a large heritability for FPR (h²=0.69 to 0.79), further indicating its reduced reliability for the evaluation of milk fat depression occurrence. According to Oetzel (2007, 2012), milk fat depression in Holstein is broadly defined as a milk fat test below 3.2% and normal milk fat percentage for Holstein herds is between about 3.4 and 4 percent. Moreover, fat tests lower than 2.5% should not be present in more than about 10% of the cows tested in Holstein herds, and typically in animals between 30 and 70 days in milk (DIM). Data from Johnston and Murphy (1984) seems to indicate some significant improvements of the technological properties of milk at levels of fat between 3 and 3.5%. In a study conducted on 118 dairy herds in northern Italy, Malacarne et al. (2014) showed optimal and suboptimal lactomyographic classification in milk containing more than 3.6% fat — and in general the highest content of dry matter and its constituents-, suggesting the adequacy of these levels of fat also for milk processing.

Despite the wide availability of data regarding individual milk fat, this information is often underestimated on a farm scale, potentially leading to economic losses and poor herd management.

Milk fatty acids (FA) profile has been proposed as a potential diagnostic tool of rumen function, as FA concentration in milk widely depends on ruminal bacteria populations (Vlaeminck et al., 2006a). The odd- and branched-chain FA of milk (OCFA and BCFA, respectively) are mainly derived from the membrane lipids of ruminal amylolytic and cellulolytic bacteria, respectively. Thus, changes in cow diet can affect their concentrations (Vlaeminck et al., 2006a; Colman et al., 2012). Furthermore, a decrease in rumen pH related to the presence of highly fermentable carbohydrates in the diet can induce a shift in the biohydrogenation of dietary unsaturated FA (in particular C18:2n-6) from C18:1trans11 and conjugated linoleic acid (CLA) cis9trans11 pathway to C18:1trans10 and CLAtrans10cis12 pathway (Bauman and Grinari, 2003), with the occurrence of MFD. These modifications often occur in fresh cattle fed high energy concentration and low roughage diet when they are not adequately adapted to the high volatile fatty acid (VFA) loads (Stone, 2004). They can lead to variations in mammary gland synthesis manifested as MFD and can induce hidden production losses related to impaired efficiency and metabolic disorders (Krause and Oetzel, 2007, 2012).
Milk fat depression, yield and fatty acid

2005). However, the larger part of the studies on this topic are confined, controlled studies, not fully representative of the real conditions of the herds.

We hypothesize that the occurrence of MFD in early lactation cattle of commercial dairy herds could impact the subsequent lactation performances and milk fat characteristics and could be considered as an indicator of potential efficiency losses of the dairy enterprise.

The objectives of this field study were to investigate the productivity of cattle identified to have MFD during early lactation in three commercial herds in Northern Italy and to study the relationship between MFD and FA composition under field condition in the attempt to investigate the origin of MFD in the commercial herds.

Materials and methods

Description of the farms/herds

Three Holstein dairy herds located in the Po plain (Northern Italy), specifically in the provinces of Modena (44°47'35.21''N; 10°57'05.28''E) and Parma (44°49'46.52''N; 10°15'24.49''E) and 44°40'41.88''N; 10°01'35.80''E), were selected for the study. The Farms (A, B, and C) had 753, 480, and 192 lactating cattle housed in free stalls and applied standard operating procedures for most important herd management processes (e.g. grouping strategies, reproduction management, veterinary practices). Cattle were milked and fed total mixed ration (TMR) twice a day on all the farms. During the observation period, which included the first six months of lactation, the animals of each farm received the fresh cow diet, whose formulation and composition was substantially constant (Tables 1 and 2). Reproduction was carefully managed and parturitions were distributed evenly throughout the study period. As reported in Table 1, the diets on Farms A and C were comprised by only dried forages, such as alfalfa and grass hays. The diet on Farm B was based on corn silage, alfalfa baleage and alfalfa hay. Ground corn was used as main source of starch in all diets. All the farms supplemented the diets with commercial concentrate mixes, which were verified to meet the requirements of cattle of the different herds for Metabolizable Energy (ME) and Metabolizable Protein (MP), such as for minerals and vitamins, relative to their actual average milk yield (ME and MP balances equal to zero for milk yields of 34, 37 and 34 kg in farm A, B and C respectively). Diets were evaluated using the CNCPS model ver. 6.1 (Cornell University, Ithaca, NY, USA).

Cow measurements and sampling

The study was conducted on a total of 950 multiparous cattle (2nd and 3rd lactation cattle) that calved in the period between January 1st, 2009 and December 1st, 2010. The individual milk production was recorded starting from day 10 after parturition every two weeks for the first six months of lactation, and individual milk was sampled monthly for fat and true protein content determination on each farm. Individual average milk yield, fat and true protein contents, such as the prevalence of cattle with MFD (milk fat lower than 3.2%) and severe milk fat depression (SMFD; milk fat lower than 2.5%) were calculated and expressed on a monthly base. Data regarding cattle of the observed herds were subsequently elaborated separately on the basis of the presence/absence of MFD according to the values recorded during the first two months of lactation, creating two virtual groups: cattle with milk fat lower than 3.2 [milk fat depression (MFD)] and cattle with fat higher than 3.2 [not milk fat depression (NMFD)] in the first two months of lactation. Individual lactation dynamic of milk fat and protein contents, FPR, milk yield and energy corrected milk (ECM), were monitored within the two groups during the first six months of lactation. The ECM was calculated according to Tyrrell and Reid (1965): ECM = 0.327 * milk lbs. + 12.97 * fat lbs. + 7.21 protein lbs and the values were then converted to kg. Fat corrected milk was calculated according to Hjulens (2010) as 3.5% FCM = (0.432 x lb of milk) + (lb of fat x 16.23) and the values were then converted to kg. Milk yield and ECM lactation curves parameters (a, b, c, Peak week, Peak yield and Persistency) were estimated for the two fat groups of cattle (MFD and NMFD) according to the Wood’s algebraic model of the lactation curves in cattle (Wood, 1967). Samples of 16 NMFD cattle and 16 MFD cattle were then selected within each herd, and a total of 96 individual milk samples were collected at the second month of lactation for milk fatty acid analysis. Samples of NMFD and MFD cattle were composed of an equal number of 2nd and 3rd lactation cows in the second or third month of lactation, showing a BCS between 2.75 and 3.00 that gave parturition in the months of February or March.

At the end of the observation period, the final yearly report on herd performances from the national breeders association (Associazione Italiana Allevatori (AIA)) was consulted for each farm in the attempt to obtain information regarding herd efficiency and performances.

Diet sampling and analyses

The TMR samples were collected twice a month, divided into two subsamples, and stored at –20°C prior to analyses. Forage samples were collected periodically to balance the diet according to the variations in forage quality. The TMR dry matter (DM) content was determined by oven drying at 55°C to constant weight. The first TMR subsample was ground in a Cyclotec mill (Tecator Inc., Herndon, VA, USA) to pass a 1-mm screen and analyzed for CP and ether extract (EE), according to AOAC (2005). The ash content was determined by ignition to 550°C; NDF, ADF and lignin con-

Table 1. Ingredients of the diets fed to the lactating cattle in farms A, B and C.

| Ingredients              | Farm A | Farm B | Farm C |
|--------------------------|--------|--------|--------|
| Grass hay                | -      | -      | 7.4    |
| Alfalfa hay              | 34.6   | 13.2   | 33.1   |
| Mixed hay                | 5.8    | -      | -      |
| Corn silage              | -      | 31.9   | -      |
| Alfalfa baleage          | -      | 4.0    | -      |
| Straw                    | -      | -      | 3.7    |
| Corn ground              | 28.9   | 14.1   | 13.3   |
| Corn flakec              | -      | 9.2    | -      |
| Barley ground            | 10.9   | -      | -      |
| Concentrate*             | 19.8   | 22.1   | 42.4   |
| Cottonseed               | -      | 5.5    | -      |

*Concentrate of farm A (% of DM) is as follows: soybean hulls, 28.0; beet pulp, 24.8; soybean flaked, 11.0; corn meal, 9.6; molasses, 9.0; soybean meal, 4.0; wheat bran, 3.8; sunflower meal, 2.0; vitamin mineral premix, 7.8. Concentrate of farm B (% of DM) is as follows: soybean hulls, 34.0; beet pulp, 32.4; soybean meal, 16.6; sunflower meal, 8.3; vitamin mineral premix, 8.7. Concentrate of farm C (% of DM) is as follows: wheat bran, 21.5; barley ground, 22.0; beet pulp, 22.0; corn ground, 19.0; soybean meal, 5.6; sunflower meal, 5.0; vitamin-mineral premix, 4.5.
tents were determined using the methods described by Van Soest et al. (1991) with the use of amylase in the NDF procedure; a semi-automated system was employed for the boiling and filtration phase (FIWE Raw Fibre Extractor, VELP Scientifica, Usmate Velate, Italy). The starch concentration was determined according to the methods of the AOAC (2005), using a K-AMYL assay kit (Megazyme International Ireland, Bray, Ireland). Non-fibre carbohydrate was calculated by difference: 100-(%NDF+%CP+%EE+%Ash). Dietary total unsaturated FA levels were estimated evaluating the diet using the software CNCPS ver. 6.1 at each new ration adjusting procedure that followed forage sampling and analysis. In vitro NDF digestibility (NDF-D) was measured at 24 and 48 hours, as described by Goering and Van Soest (1970), using rumen fluid collected by esophageal probe from a dry cow fed two kg of concentrate per day and given ad libitum access to grass/alfalfa mixed hay (55% NDF; 14% CP). The rumen fluid collection procedure complied with Italian laws on animal welfare.

The second TMR subsample was used to measure the particle size using the Penn State Particle Separator (PSPS), as described by Lammers et al. (1996); a dry sieving procedure was adopted considering the results on sieving at the different moisture levels reported by Kononoff et al. (2003). The physically effective NDF (peNDF) 8.0 and peNDF 1.18 were estimated by multiplying the NDF concentration of the TMR by the proportion of particles >8.0 mm and >1.18 mm, respectively (Kononoff et al., 2003; Beauchemin and Yang, 2005).

**Milk sampling and analyses**

The milk fat and true protein contents were determined monthly on individual milk samples of cattle of the whole herds. Moreover, fat, protein, caseins, lactose and urea contents and FA composition analyses were conducted on individual milk samples taken from the NMFD selected cattle (n=16) and from the MFD selected cattle (n=16) on each farm. These samples, composed as a proportional mix of the morning and evening milking, were transported to the laboratory, divided into two sub-samples and stored at 4°C. The milk fat, protein, lactose, urea, and casein contents were measured on a subsample by Fourier transformed infrared spectroscopy (MilkoScan FT6000; Foss System, Hillerød, Denmark). The other subsample was frozen until FA analysis. Before analysis, samples were kept at room temperature for 12 h, then centrifuged at 4°C and 3700 rpm for 15 min to separate the cream. The cream was centrifuged at 35°C and 20000 rpm for 35 min. The supranatant anhydrous fat was separated and directly analyzed for FA. The FA trans-esterification was obtained using Sodium-methylate 2M to start and sodium sulfate monohydrate to stop the methylation processes, according to Revello-Chion et al. (2010). The FA methyl esters (FAME) gas-chromatographic analysis was performed as described by Coppa et al. (2011). A reference standard butter (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to estimate correction factors for the short-chain FA (C4:0 to C10:0).

**Table 2. Chemical composition and particle size distribution, measured using the Penn State Particle Separator, of the total mixed ration fed to the lactating cattle on the studied farms.**

| Item                                | Farm A | Farm B | Farm C | SEM  | Significance |
|-------------------------------------|--------|--------|--------|------|--------------|
| **Chemical composition**            |        |        |        |      |              |
| DM                                  | 50.8   | 45.9   | 50.8   | 0.70 | *            |
| Ash (% of DM)                       | 7.17   | 6.37   | 7.81   | 0.25 | ***          |
| CP (% of DM)                        | 16.3   | 16.0   | 15.0   | 0.18 | *            |
| EE (% of DM)                        | 2.29   | 3.69   | 3.19   | 0.14 | ***          |
| Sugars + Pectins (% of DM)          | 14.9   | 11.0   | 14.5   | 0.97 | ns           |
| Starch (% of DM)                    | 26.3   | 27.2   | 21.8   | 0.76 | †            |
| NFC (% of DM)                       | 41.2   | 38.3   | 36.3   | 0.86 | ***          |
| NDF (% of DM)                       | 33.0   | 35.6   | 37.8   | 0.61 | ***          |
| ADF (% of DM)                       | 18.6   | 19.1   | 21.2   | 0.57 | †            |
| ADL (% of DM)                       | 4.43   | 3.67   | 4.59   | 0.15 | *            |
| NDF-D 24h (% of NDF)                | 49.5   | 46.1   | 48.6   | 1.38 | ns           |
| NDF-D 48h (% of NDF)                | 53.7   | 56.7   | 53.3   | 1.26 | ns           |
| dNDF 24h (% of DM)                  | 16.3   | 16.4   | 18.4   | 0.48 | ns           |
| dNDF 48h (% of DM)                  | 17.7   | 20.2   | 20.1   | 0.41 | **           |
| P:C                                 | 0.68   | 0.96   | 0.79   | 0.04 | ***          |
| Total unsaturated FA (%DM)°         | 1.78   | 1.53   | 1.85   | 0.06 | *            |
| NEI (Mcal/kg)°                      | 1.59   | 1.56   | 1.45   | 0.04 | ***          |
| **Particle size analysis**          |        |        |        |      |              |
| > 19 mm                             | 6.82   | 7.14   | 10.3   | 0.56 | ***          |
| 19.0-8.0 mm                         | 17.1   | 35.0   | 19.7   | 1.82 | ***          |
| 8.0-1.18 mm                         | 51.9   | 31.2   | 37.7   | 2.00 | ***          |
| < 1.18 mm                           | 24.2   | 26.6   | 32.3   | 1.02 | *            |
| peNDF1.8                            | 7.84   | 15.0   | 11.3   | 0.80 | ***          |
| peNDF1.18                           | 25.2   | 26.1   | 25.6   | 0.31 | ns           |

DM, dry matter; CP, crude protein; EE, ether extract; Sugars + Pectins, NFC-starch, NFC, non fibrous carbohydrate; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NDF-D, in vitro NDF digestibility; dNDF, digestible NDF; F:C, forage to concentrate ratio. Values estimated using the CNCPS model ver. 6.1. °Determined using the Penn State Particle Separator (PSPS), dry sieving; peNDF8.0, physically effective NDF, ration NDF multiplied by amount of DM > 8.0 mm; peNDF1.18, physically effective NDF, ration NDF multiplied by amount of DM > 1.18 mm. *P<0.05; **P<0.01; ***P<0.001; ns, not significant.
Statistical analysis

Statistical analyses were performed using the SPSS for Windows software package (SPSS Statistics for Windows, ver. 17.0. Chicago, IL, USA; SPSS, 2008). The diets chemical composition parameters were compared through the general linear model, univariate analysis procedure, using the LSD as post hoc test. The milk chemical composition and cow performance data of the entire dairy herds were processed using a mixed model of the analysis of variance, using the interval as a repeated factor and the farm and the MFD group as fixed factors. Interactions farm × group, farm × interval, interval × group and farm × group × interval effects were also included in the model. Data on the selected cattle performance and on the related milk chemical and FA composition were processed using the general linear model of analysis of variance, in which the MFD group, the farm and the group x farm interaction were used as fixed factors. The cow was used as the statistical unit in each analysis. Milk yield and ECM lactation curves parameters were calculated using the algebraic model of the lactation curves in cattle (Wood, 1967) applying the non-linear regression procedure of the SPSS for Windows software package.

Results

Diet composition

The average formulation and the chemical and physical characteristics of the diets adopted in the farms are reported, expressed as percent of DM, in Tables 1 and 2. NDF was 33.0, 35.6 and 37.8% in Farms A, B and C, (P<0.001) while NFC levels were 41.2, 38.3 and 36.3%, respectively, with significant differences between farms (P<0.001). A trend for a significant difference was observed between starch levels, which were 26.3, 27.2 and 21.8%, representing the 63.8, the 71.0 and the 60.1% of NFC in farm A, B and C, respectively. The CP content was higher on Farm A than on Farm C (16.3 vs 15.0% of DM; P<0.05) while Farm B showed an intermediate value (16.0% of DM). The EE was significantly lower in Farm A than in Farms B and C, respectively; P<0.001) and Farm C showed the intermediate value (16.3% of DM). The dNDF at 24 hours (dNDF 48h) ranged from a minimum of 16.3% (DM basis – Farm A) to a maximum of 18.4% DM (Farm C). The dNDF at 48 hours (dNDF 48h) ranged from a minimum of 17.7% DM on Farm A to a maximum of 20.2% DM on Farm B and was significantly lower in Farm A (P<0.01).

The particle size distributions of the TMR fed on the studied farms are given in Table 2. The material retained by the 19.0 mm screen was greater for Farm C than for Farms A and B, whereas the 19.0 to 8.0 mm particles were greater in the TMR of Farm B than on those of Farms A and C. The proportion of 8.0 to 1.18 mm particles was greater on Farm A than on Farm B and C, with intermediate values on Farm C. Those differences were highly significant (P<0.001). Conversely, the proportion of particles below 1.18 mm was greater on Farm C than on Farm A, with intermediate values on Farm B. The peNDF 1.18 mm, did not differ among the TMR of the studied farms, while the peNDF 8.0 mm showed greater values on Farm B and lower values on Farm A with an intermediate value on Farm C.

Severe milk fat depression and milk fat depression occurrence, milk yield and milk composition at farm level

During the observation period, the prevalence of SMFD was found to be significantly higher in Farm A than in Farm C (5.0 vs 1.5%; P<0.001) with an intermediate value in Farm B whose average resulted significantly higher than the average of Farm C (3.9 vs 1.5%; P<0.01). In Farm A, SMFD prevalence ranged from 3.3 to 6.7% while in Farm B and C this parameter ranged from 3.1 to 5.5% and from 0 to 3.1%, respectively. The highest prevalence of MFD milk was recorded in Farm A, with an average value of 33.1% (ranging from 10.7% to 43.0% over the experimental period). Farm B showed the intermediate average prevalence of MFD milk (27.0%), ranging from 12.3% to 32.5%, whereas the incidence of MFD milk on Farm C ranged from 7.5% to 36.1%, with an average proportion of 17.6% during experimental period. The difference between Farm A and C was significant with P<0.01 while the difference between Farm B and C was significant with P<0.05. The difference between Farm A and B was not significant.

The main effects of fat groups, farm and interval on milk yield, which was affected also by farm and by interval (P<0.001) and the differences observed between MFD and NMFD groups were consistent between farms. In particular, MFD milk showed a significantly higher average milk yield than NMFD milk and the average difference ranged from a minimum of 1.1 kg of Farm C to a maximum of 2.7 kg of Farm B. The variation of milk yield over time was different (P<0.001) between groups; starting from the 3rd month of lactation, the MDF group in Farm C showed a lower milk yield.

The ECM appeared to be significantly affected both by farm and interval (P<0.001), while only a trend was observed when group was the independent variable (P<0.1). This parameter resulted constantly lower in MFD than in NMFD milk in Farm A and C, while in Farm B the trend was the opposite starting from the 3rd month of lactation, leading to the same mean value in the two groups at the end of the first 6 months of lactation.
Table 3. Fat and protein milk contents, milk fat to protein ratio, milk yield and energy corrected milk, during the first 6 months of lactation in farms A, B and C for milk fat level groups.

| Item                      | Interval | Mean | SEM | Effect and significance |
|---------------------------|----------|------|-----|-------------------------|
|                           | 1       | 2    | 3   | 4   | 5   | 6   | G   | F   | I   | G × F | G × I | F × I | G × F × I |
| Fat, g/100 g milk         |          |      |     |     |     |     | 0.006 |     |     |     |     |     |     |
| Farm A                    |          |      |     |     |     |     | ***  | ns  |     |     |     |     |     |
| MFD                       | 2.99     | 2.83 | 3.11| 3.23| 3.31| 3.47| 3.16  |     |     |     |     |     |     |
| NMFD                      | 3.95     | 3.56 | 3.50| 3.54| 3.68| 3.75| 3.66  |     |     |     |     |     |     |
| Farm B                    |          |      |     |     |     |     |     | ***  | **  |     |     |     |     |
| MFD                       | 3.02     | 2.87 | 3.12| 3.31| 3.40| 3.49| 3.20  |     |     |     |     |     |     |
| NMFD                      | 4.09     | 3.63 | 3.58| 3.59| 3.65| 3.73| 3.71  |     |     |     |     |     |     |
| Farm C                    |          |      |     |     |     |     |     |     |     | *** |     |     |     |
| MFD                       | 2.93     | 3.02 | 3.29| 3.47| 3.49| 3.58| 3.30  |     |     |     |     |     |     |
| NMFD                      | 3.79     | 3.51 | 3.60| 3.72| 3.82| 3.85| 3.72  |     |     |     |     |     |     |
| Protein, g/100 g milk     |          |      |     |     |     |     | 0.003 |     |     |     |     |     |     |
| Farm A                    |          |      |     |     |     |     | ***  | **  | *** |     |     |     |     |
| MFD                       | 3.25     | 3.19 | 3.33| 3.43| 3.53| 3.61| 3.39  |     |     |     |     |     |     |
| NMFD                      | 3.41     | 3.27 | 3.36| 3.47| 3.57| 3.64| 3.45  |     |     |     |     |     |     |
| Farm B                    |          |      |     |     |     |     |     |     | *** | **  |     |     |     |
| MFD                       | 3.08     | 3.02 | 3.14| 3.23| 3.30| 3.35| 3.19  |     |     |     |     |     |     |
| NMFD                      | 3.26     | 3.11 | 3.22| 3.34| 3.40| 3.45| 3.30  |     |     |     |     |     |     |
| Farm C                    |          |      |     |     |     |     |     |     |     |     | *** |     |     |
| MFD                       | 3.03     | 3.12 | 3.30| 3.45| 3.47| 3.57| 3.32  |     |     |     |     |     |     |
| NMFD                      | 3.27     | 3.30 | 3.45| 3.51| 3.63| 3.68| 3.47  |     |     |     |     |     |     |
| FPR                       |          |      |     |     |     |     | 0.002 |     |     |     |     |     |     |
| Farm A                    |          |      |     |     |     |     | ***  | *** | *** |     |     |     |     |
| MFD                       | 0.93     | 0.89 | 0.94| 0.94| 0.96| 0.96| 0.93  |     |     |     |     |     |     |
| NMFD                      | 1.17     | 1.10 | 1.04| 1.02| 1.03| 1.03| 1.07  |     |     |     |     |     |     |
| Farm B                    |          |      |     |     |     |     |     |     | *** | *** |     |     |     |
| MFD                       | 0.99     | 0.95 | 1.00| 1.03| 1.03| 1.03| 1.01  |     |     |     |     |     |     |
| NMFD                      | 1.26     | 1.17 | 1.11| 1.00| 1.01| 1.01| 1.13  |     |     |     |     |     |     |
| Farm C                    |          |      |     |     |     |     |     |     |     |     | *** |     |     |
| MFD                       | 0.98     | 0.97 | 1.00| 1.01| 1.00| 1.01| 0.99  |     |     |     |     |     |     |
| NMFD                      | 1.16     | 1.07 | 1.05| 1.06| 1.05| 1.04| 1.07  |     |     |     |     |     |     |
| Milk yield, kg/cow × day  |          |      |     |     |     |     | 0.07  |     |     |     |     |     |     |
| Farm A                    |          |      |     |     |     |     | ***  | *** | *** | ns  | *** | *** |     |
| MFD                       | 37.2     | 39.2 | 36.9| 35.0| 32.1| 30.2| 35.1  |     |     |     |     |     |     |
| NMFD                      | 33.1     | 37.1 | 36.0| 33.8| 31.5| 29.8| 33.5  |     |     |     |     |     |     |
| Farm B                    |          |      |     |     |     |     |     |     | *** | *** |     |     |     |
| MFD                       | 38.5     | 41.7 | 41.3| 39.3| 36.9| 34.8| 38.7  |     |     |     |     |     |     |
| NMFD                      | 33.7     | 38.7 | 38.4| 37.1| 35.0| 33.0| 36.0  |     |     |     |     |     |     |
| Farm C                    |          |      |     |     |     |     |     |     |     |     | *** |     |     |
| MFD                       | 40.3     | 39.0 | 36.4| 31.8| 28.1| 25.8| 33.5  |     |     |     |     |     |     |
| NMFD                      | 36.0     | 36.9 | 35.0| 31.4| 29.1| 26.0| 32.4  |     |     |     |     |     |     |
| ECM, kg/cow × day         |          |      |     |     |     |     | 0.07  |     |     |     |     |     |     |
| Farm A                    |          |      |     |     |     |     | ***  | *** | *** |     | ns  | *** |     |
| MFD                       | 35.2     | 36.0 | 35.6| 34.5| 32.2| 31.0| 34.1  |     |     |     |     |     |     |
| NMFD                      | 35.6     | 37.7 | 36.6| 34.7| 33.2| 31.7| 34.9  |     |     |     |     |     |     |
| Farm B                    |          |      |     |     |     |     |     | ***  | *** | *** |     |     |     |
| MFD                       | 35.7     | 38.1 | 39.4| 38.7| 36.9| 35.3| 37.3  |     |     |     |     |     |     |
| NMFD                      | 36.5     | 39.4 | 39.1| 38.0| 36.3| 34.6| 37.3  |     |     |     |     |     |     |
| Farm C                    |          |      |     |     |     |     |     |     |     |     | *** |     |     |
| MFD                       | 37.4     | 36.6 | 35.9| 32.7| 28.7| 26.9| 33.0  |     |     |     |     |     |     |
| NMFD                      | 37.7     | 37.4 | 36.1| 33.1| 31.3| 28.3| 34.0  |     |     |     |     |     |     |

G, fat group; F, farm; I, interval; MFD, cattle with fat<3.2%; NMFD, cattle with fat>3.2%; FPR, fat to protein ratio; ECM, energy corrected milk. °Interval, month of monitoring after calving; values between interval, groups and farm with different superscript letters are different at P<0.05 (interaction G × F × I); †mean value per farm x fat level group, values between groups and farm with different superscript letters are different at P<0.05 (interaction G × F). *P<0.05; **P<0.01; ***P<0.001; †P<0.1; ns, not significant.
months of lactation. The ECM of the MDF cattle averaged 34.1, 37.3 and 33.0 kg, while the ECM of NMFD cattle resulted 34.9, 37.3 and 34.0 kg respectively in Farm A, B and C. A significant interaction was observed between farm and interval (P<0.001), indicating the different ECM production dynamic of the farms. Independently from groups, cattle in Farm B showed a higher ECM production.

Milk yield Wood’s lactation curve parameters were a=35.0 and 27.1; b=0.146 and 0.255; c=0.023 and 0.028 for MDF and NMDF groups, respectively; in the same groups, cattle were estimated to peak at 6.3 and 9.1 weeks with peak yields of 39.6 and 36.8 kg and persistence of 75.4 and 88.9%. For ECM, the same lactation curve parameters in NMFD and MFD groups were a=31.1 and 30.2; b=0.178 ad 0.179; c=0.022 and 0.021; MDF cattle were estimated to peak at 8.5 weeks, with a peak yield of 37.0 kg and a persistency of 95.1% while NMDF cattle were estimated to peak on average at 8.1 weeks, with a peak yield of 37.7 and a persistency of 89.7%. The estimated dynamic of milk yield and ECM production are represented in Figures 1 and 2.

The final yearly report on herds’ performances from the national breeders association (AIA – Associazione Italiana Allevatori, Roma, Italy) showed a lower milk yield per lactation in Farm A (9224±2085 kg) with respect to Farm B (10783±2178 kg) and C (10772±1831 kg). Average daily production was 30.2 kg in Farm A, while it was 34.0 kg and 34.1 kg in Farm B and C, respectively. Average fat percentage was 3.50, 3.64 and 3.47%, while protein percentage was 3.58, 3.38 and 3.36% in Farm A, B and C, respectively. Culling rate was similar in the three considered farms, being 34, 32 and 34% in Farm A, B and C, respectively. Only on average, cattle from Farm A were culled earlier (132 DIM) than cattle in Farm B (144 DIM) and the latter were culled earlier than cattle in Farm C (157 DIM).

Milk yield, milk composition and fatty acid profile at selected animal level

Data regarding milk yield and composition of the selected animals of each farm are summarized in Table 4 for the fat groups and farms. Milk yield and the ECM did not significantly differ between the two groups of cattle. A significant difference was found for FCM (P<0.01), fat level and FPR (P<0.001). Protein level was tendentially higher in the MFD group (P<0.1).

Differences between farms were observed for protein level, FPR and DIM (P<0.01); only trends were observed for milk yield, FCM and these parameters between groups in the different farms.

The FA composition of the milk fat of the selected cattle for the fat groups and farms is given in Table 5 for individual FA and in Table 6 for the sums and ratios of FA. The milk of the MFD cattle had greater (P<0.05) concentrations of C10:0, C12:0, C13:0, C14:0,
and C21:0 were observed on Farm A when compared with Farm B and C. Some significant values of saturated fatty acids, OCFA, total de novo preformed FA, and of the OCFA/BCFA, C18:1trans9, tend to have greater concentrations in the milk of the MFD cattle than in the milk of the NMFD cattle (P<0.1); an opposite trend was observed for isoC18:0, C18:0, C18:1trans11 and C18:1cis11 and C23:0, total MUFA, and total C18:1cis isomers than the NMFD cattle. The C7:0, C11:0, C12:1cis9, C14:1cis9, C14:1cis9, C18:1trans6/7/8, and C18:1trans9, tended to have greater concentrations in the milk of the MFD cattle than in the milk of the NMFD cattle (P<0.1); an opposite trend was observed for isoC18:0, C18:0, C18:1trans11 and C18:2cis9trans13 (P<0.1). The effect of the farm was significant for almost all the milk FA, except for C4:0, C14:1trans9, anteisoC17:0, C16:1cis11, C18:1trans13, C19:0, C18:1cis16, C22:0, C22:3, and C24:0. In particular, a general differentiation trend was observed for Farm A in comparison to Farms B and C. Farm A showed higher values of saturated fatty acids, OCFA, total de novo preformed FA, and of the OCFA/BCFA, C18:1trans10/C18:1trans11 and C14:1cis9/14:0 ratios. In analogy with the variations observed in the MFD cattle, higher values of C7:0, C11:0, C12:0, C12:1cis9, C13:0, C14:1cis9, C15:0, C22:3n-3+C24:1, C20:4n-6 and C22:3n-3+C24:1cis9 and lower levels of C18:0 (trend), C18:1trans11 (trend), C18:1cis9 and C21:0 were observed on Farm A when compared with Farm B and C. Some significant interactions between farm and group were also observed; the most evident were related to C7:0, C11:0, C13:0 C18:1trans12+6 and C18:2cis9trans13, such as for odd chain fatty acids (OCFA).

Discussion

The purpose of this study was to evaluate the impact of MFD on dairy cattle milk production on three commercial dairy farms and to measure the changes in milk FA composition in relation to the presence/absence of MFD in order to investigate the possible origin of this phenomena under field conditions.

The DM content of the diets was in general low and significantly lower in Farm B than in Farm A and C since about 1/3 of the DM in Farm B diet was from corn silage. In Farm A and C the addition of water (about 20 kg/cow) increased moisture ensuring a homogeneous intake of the diet, also improved by the good level of comminution of the particles of the TMRs. Silage presence has also led to the higher presence of particles <19 mm and >8 mm in diet B; these particles were likely ground by the mixer and shifted to the lower pan in diets A and B, based on hay forages characterized by a fragile structure. CP showed the higher value in Farm A and the lowest in Farm C, and this could have positively affected, together with the presence of highly fermentable carbohydrates, the production of ruminal microbial proteins and the numerically higher milk protein level in milk from Farm A. The EE resulted similar in Farms B and C whose higher dietary level of fat was likely related either to the amount of soybean, corn and/or wheat bran used to formulate the diet. Estimated dietary levels of unsaturated FA were in general constantly low and under levels demonstrated to be not detrimental or potentially detrimental (3.6 to 4% DM) for mammary fat synthesis (Leonardi et al., 2005; Griniari et al., 1998; Boerman and Lock, 2014). Therefore, their presence was not considered as a variable affecting milk fat levels in the studied farms. Farms A and B diets had a similar starch concentration – higher than Farm C diet - but corn silage was used only in Farm B potentially leading to an higher NFC degradability in this diet. However, as described in Table 1, in Farm A about 1/3 of the dietary starch was from barley and wheat bran and in Farm B the starch from corn silage amounted to about 1/3 of the total starch while the 2/3 were from corn meal in both farms. Therefore, starch in the two diets differed only for the source of the secondary readily fermentable starch fraction, represented by barley and corn silage, respectively which have been estimated to have a very similar behavior in dairy cattle gastrointestinal tract (Moharrey et al., 2014). Moreover, Farm A diet contained also a considerable amount of highly fermentable sugars from molasses. The NFC level resulted significantly higher in Farm A than in Farm B and C, while the NDF of Farm A diet was significantly lower than the NDF of the other diets. The NDF content of the Farms B and C diets was higher than the maximum level recommended by Chase and Overton (2004) -33% DM- and by Sniffen (2004) -32% DM- for lactating dairy cattle. Conversely, it should be noted that the NFC level in the diet fed to fresh cattle on Farm A was higher than the maximum (40% DM) indicated by Chase and Overton (2004) for lactating dairy cattle and close to the maximum recommendations (45% DM) of Sniffen (2004), indicating a potential acidogenic action of this diet.

According to the recommendation of Zebeli et al. (2012), a minimum amount of 31.2% of MFD, above a minimum of 1.18 mm or 18.5% of peNDF including particles >8 mm [i.e., peNDF(>8)] in the diet (DM basis) should be

Table 4. Milk yield and composition of cattle selected for their milk fat level, according to the milk fat level group and to the farms.

| Group          | Farm          | SEM | Effect and significance |
|---------------|---------------|-----|-------------------------|
|               | MFD           | NMFD | A  | B  | C  |     | G  | F  | I  |
| Milk yield, kg/d | 40.0          | 37.1 | 38.3 | 41.3 | 36.0 | 0.96 | ns | †  | ns |
| ECM, kg/d     | 33.7          | 35.8 | 34.3 | 36.7 | 33.3 | 0.93 | ns | ns | ns |
| FCM, kg/d     | 33.6          | 38.8 | 36.0 | 39.0 | 33.6 | 0.95 | ** | †  | ns |
| Fat, g/100g milk | 2.52          | 3.81 | 3.16 | 3.20 | 3.14 | 0.077 | *** | ns | †  |
| Protein, g/100g milk | 3.27         | 3.19 | 3.35* | 3.13 | 3.20 | 0.024 | †  | *** | ** |
| Caseins, g/100g milk | 2.47         | 2.49 | 2.45 | 2.49 | 2.50 | 0.021 | ns | ns | ns |
| Lactose, g/100g milk | 4.90         | 4.89 | 4.86 | 4.89 | 4.94 | 0.026 | ns | ns | ns |
| Urea, mg/dL milk | 22.9          | 22.7 | 23.5 | 20.9 | 23.9 | 0.53 | ns | †  | ns |
| PPR           | 0.77          | 1.20 | 0.95 | 1.03 | 0.99 | 0.025 | *** | ** | ** |
| DIM           | 71            | 72   | 69   | 61b  | 84  | 2.9 | ns | ns | ns |

MFD, cattle with fat<3.2%; NMFD, cattle with fat>3.2%; G, group; F, farm; I, interaction; ECM, energy corrected milk; FCM, fat corrected milk; PPR, milk fat to protein ratio; DIM, days in milk. *P<0.05;
| Group | Farm | SEM | Effect and significance |
|-------|------|-----|-------------------------|
| C20:5n-3 | C20:5n-3+C22:1 | 17.93 | 20.08 |
| C14:0 | C14:0 | 11.41 | 10.43 |
| C12:1 | C12:1 | 0.04 | 0.02 |
| C14:1 | C14:1 | 0.05 | 0.03 |
| C16:0 | C16:0 | 2.09 | 2.03 |
| C18:1 | C18:1 | 0.06 | 0.04 |
| C20:2n-6 | C20:2n-6 | 0.08 | 0.07 |
| C20:3n-3 | C20:3n-3 | 0.12 | 0.11 |
| C20:4n-6 | C20:4n-6 | 0.09 | 0.08 |
| C22:1 | C22:1 | 0.16 | 0.15 |
| C22:2 | C22:2 | 0.09 | 0.08 |
| C22:3 | C22:3 | 0.09 | 0.08 |

Table 5. Fatty acid composition of the milk fat from selected cattle for their milk fat level according to the milk fat group, and to the farm.
guaranteed in order to maintain rumen physiology. Moreover, based on the same author findings, a proportion of pNDF (>8) in the diet beyond 14.9% of DM could also reduce DM intake level. In the studied farms, the minimum requirements for pNDF inclusive of particles >1.18 mm and for pNDF inclusive of particles >8 mm were never reached and the value of pNDF inclusive of particles >8 mm in Farm A was less than half of the minimum recommended values, further suggesting the acidic condition of this diet.

During the observation period, Farm A and B showed a greater prevalence of SMFD than C. This finding could be related to a larger amount of rapidly fermentable carbohydrates (starch in particular) in the diet, such as to a lower amount of NDF and, in the case of Farm A, a very low level of pNDF (Zebeli et al., 2012). Several studies (Oba and Allen, 2003; Taylor and Allen, 2005) have demonstrated that a high content of rapidly fermentable starch in rumen decreases the milk fat content, which can also be affected by the interaction between the NDF concentration and NDF digestibility of the feed diet (dNDF) which resulted lower in Farm A at 48 hours of fermentation (Oba and Allen, 2000). In the present study, Farm A and B diets had lower NDF and ADF content than Farm C diet, that together with a lower F:C ratio, could have in general reduced rumination and rumen buffering. It should be noted, however, that the prevalence of SMFD was always lower than the maximum proportion – less than 10% - proposed by Oetzel (2007, 2012).

Similarly to SMFD occurrence, the MFD prevalence was higher in Farm A and B than in Farm C during the first six months of lactation. The presence of MFD during the first two months of lactation was used to create two virtual groups of cattle (MFD and NMFMD) for further study the effects of milk fat depression on cattle productivity. As previously reported, the differences in milk fat content between MFD and NMFMD groups were found to be at the maximum levels during the first two months of lactation, usually corresponding to the negative energy balance period, when diet composition and intake become more critical in determining dairy cattle metabolism (Baumgard et al., 2006). The differences in protein levels were more constant within farms during the observation period. Because of its higher variability during the experimental period, milk fat was probably the main factor determining the dynamic of FPR values.

Butchereit et al. (2010) suggested that values of FPR between 1.05 and 1.18 could be considered as normal, with the exception of milk from cows in early lactation that showed higher FPR values due to a greater metabolic load. Thus, the milk FPR of the NMFMD cattle in the present study can be considered physiological, whereas the MFD cattle had lower FPR values throughout the whole observation period with MFD cattle of farm A showing a FPR constantly lower than 1, differently than those of farms B and C (Butchereit et al., 2010; enja and Chladek 2005). This observation appears relevant, since Toni et al. (2011) reported as the multiparous cows with FPR<1.0 in early lactation produced 2.5 kg less milk per day, compared to the multiparous cows with FPR between 1.0 and 1.5.

Overall, the differences in average milk yield between MFD and NMFMD groups in Farm A and C amounted to about 1 to 1.5 kg of milk, while in Farm B this difference amounted on average to about 2.7 kg. In Farms A and B the MFD cattle showed higher milk yield during the entire observation period, while in Farm C this difference in productivity disappeared in mid-lactation, as confirmed by the interaction between group and interval and farm and interval. This could be related to the lower energy density of the diet C that did not sustain the milk synthesis in mid-lactation accordingly with data reported by Vazquez-Añón et al. (1997) in a study focusing on the effect of dietary energy on milk production in mid and late lactation. The higher energy density of Farms A and B could have positively affected milk production and the different behavior of fat groups in Farm B could be attributable to the different forage base such as to the high amount of soybean hulls and beet pulp introduced in the diet.

Data on ECM show a trend for a difference

Table 6. Sum and ratios of the milk fatty acids in response to cattle with different milk fat levels on the studied farms

|                | Group                  | Farm     | SEM   | Effect and significance |
|----------------|------------------------|----------|-------|------------------------|
|                |                        |          | F     | I                      |
| FA, g/100 g FA |                        |          |       |                        |
| SPA            | 66.72                  | 65.63    | 68.21 | 63.88                  | 66.31 | 0.418 | ns    | ***   | ns    |
| MUF A          | 26.68                  | 28.23    | 25.66 | 29.85                  | 27.19 | 0.393 | *     | **    | ns    |
| PUFA           | 5.21                   | 4.88     | 4.81  | 5.03                   | 5.11  | 0.075 | *     | *     | ns    |
| OCFA           | 2.77                   | 2.50     | 3.21  | 2.17                   | 2.49  | 0.081 | *     | ***   | **    |
| BCFA           | 1.44                   | 1.47     | 1.34  | 1.38                   | 1.64  | 0.031 | ns    | ***   | ns    |
| OCFA/BCFA      | 2.07                   | 1.76     | 2.50  | 3.55                   | 1.60  | 0.084 | *     | ***   | ***   |
| Σ trans-FA     | 4.03                   | 3.54     | 3.11  | 4.50                   | 3.79  | 0.137 | *     | **    | ns    |
| Σ C18:1 trans  | 2.90                   | 2.45     | 2.15  | 3.26                   | 2.65  | 0.119 | *     | ***   | ns    |
| Σ C18:1 cis    | 20.23                  | 22.30    | 19.29 | 23.11                  | 21.53 | 0.380 | **    | ***   | ns    |
| Σ CLA isomers  | 0.42                   | 0.42     | 0.33  | 0.47                   | 0.46  | 0.011 | ns    | ***   | †     |
| Σ de novo produced FA | 24.71                | 23.71    | 25.62 | 22.14                  | 23.95 | 0.317 | **    | ***   | ns    |
| Σ n-6          | 3.36                   | 3.10     | 3.16  | 3.33                   | 3.01  | 0.053 | ***   | ns    | ns    |
| Σ n-3          | 0.89                   | 0.86     | 0.87  | 0.62                   | 1.12  | 0.028 | ns    | ***   | ns    |
| Σ n-6/Σ n-3    | 4.12                   | 4.06     | 3.64  | 5.53                   | 2.77  | 0.158 | ns    | ***   | ns    |
| C18:1 trans/10/C18:1 trans11 | 1.24            | 0.63     | 1.46  | 0.72                   | 0.59  | 0.118 | **    | †     | ns    |
| C14:1 cis/C14:0 | 0.085                | 0.082    | 0.096 | 0.077                  | 0.076 | 0.003 | ns    | ns    | ns    |

FA, fatty acids; MFD, cattle with fat<3.2%; NMFMD, cattle with fat>3.2%; G, group; F, farm; I, interaction; SPA, saturated fatty acids; MUF A, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OCFA, odd chain fatty acids; BCFA, branched chain fatty acids; Σ de novo produced FA= C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0. *P<0.05; **P<0.01; ***P<0.001; P<0.1; ns, not significant.
in mammary energy output between fat groups in the observed farms, with lower average values in MFD groups of Farms A and C. The diet adopted in the different farms lead to different production levels and lactation curves as indicated by the significant differences found for farm and interval and the interaction between those factors. This is in agreement with data reported by Nielsen et al. (2007) indicating a variable effect of changes in diet energy density on feed intake, milk yield and cattle metabolism. The Wood's parameters describing the milk yield and ECM lactation curves of MFD and NMFD cattle groups seem in general to confirm the trend previously reported. MFD cattle have in general a higher initial milk yield, peak earlier with higher peak yield, even if they appear to have a lower lactation persistency when compared to NMFD cattle. However, the ECM curve parameters of the two groups of cattle indicate similar curve shape between groups with an higher initial ECM yield for NMFD cattle which slightly anticipat-ed the peak and showed an higher peak yield even if with lower persistency with respect to the MFD group.

Data from the final yearly report from the national breeders association regarding the observed farms indicate a lower productivity (30.2 vs 34.0 and 34.1 kg/cow/d) and a earlier culling time (132 vs 144 and 157 DIM) of cattle of the herd A, characterized by a higher prevalence of MFD, despite the similar culling rate between farms. This is consistent with data reported by Toni et al. (2011) which found a significantly higher early lactation diseases incidence in cattle showing a low FPR – probably characterized by MFD.

AlZahal et al. (2009) demonstrated that milk from cattle fed with acidogenic diets had greater concentrations of C7:0, C9:0, C10:0, C11:0, C12:1, C13:0, C15:0 than cattle fed more fibrous diets. Similar variations were observed in the milk of the MFD cattle studied in the present work.

Moreover, the data obtained in the present study, show an increase of OCFA related both to the concentrated diet adopted in the Farm A and to the group of MFD cattle. Variations of OCFA have been reported also by other authors under specific experimental conditions: Rigott et al. (2003) showed that propionate infusion in the rumen increased the proportion of OCFA in milk fat, thus supporting an effect of highly concentrated diets on these milk FA. Furthermore, the proportion of milk OCFA, particularly of C15:0, was positively and straightly related to the molar proportion of propionate in the rumen in a study from Vlaeminck et al. (2006b). Enjalbert et al. (2008) demonstrated that cattle with induced sub-acute ruminal acidosis (SARA) showed an increase in milk OCFA concentration, and in particular in C11:0, C13:0 and C15:0 concentrations. Vlaeminck et al. (2006a) highlighted a positive relationship between OCFA and the abundance of amyloptic bacteria and between BCFA and the abundance of cellulolytic bacteria in the rumen, and this appear consistent with the finding reported in our study, where an increase of the OCFA/BCFA ratio was shown in the high concentrate diets. It should be underlined that the same variation was also found in cattle showing MFD. Based on Craninx et al. (2007), that proposed the use of OCFA and BCFA concentrations in milk to diagnose acute acidosis and SARA in dairy cattle, the increase of OCFA in MFD group could be explained. The interaction between group and farm for OCFA could be the result of a different effect of Farm B diet, which contained corn silage as a main forage source and high levels of non-forage fibre sources. Bauman and Griniari (2005), and Enjalbert et al. (2008) demonstrated that a reduction in ruminal pH, induced by SARA, can affect the ruminal bacteria that are responsible for dietary PUFA bihydrogenation. In particular, a shift from the CLAcis9trans11 and C18:1trans11 bihydrogenation pathways to the CLAcis11cis12 and C18:1trans10 pathways was observed in the rumen of acidotic cattle, which induce an increase in the C18:2n-6 and C18:1trans-10 leaving the rumen and transferred to the mammary gland were they act as strong inhibitors fat synthesis. Both of these fatty acids were increased in our study and according to Bauman and Griniari (2005), a probable depressing effect on milk fat induced by C18:1trans10 was observed in MFD cattle that showed a higher concentration of this FA and a higher C18:1trans10/ C18:1trans11 ratio. It must be emphasized that the increase of C18:1trans10/C18:1trans11 ratio was found in milk by Enjalbert et al. (2008) in association with a low ruminal pH, inducing the authors to suggest that these FA could be used to diagnose acute acidosis and SARA in dairy cattle. In the present study, unsaturated fatty acids were present at medium levels, and the occurrence of acidogenic factors could have induced the biochemical variations previously described, leading to MFD.

Considering the hypothesis that the differences in the FA profile of milk between MFD and NMFD cattle could be related to the occurrence of some individual factor within MFD group leading to acidic condition, it is also possible to explain the greater level in some specific FA (such as C7:0, C9:0, C11:0, C12:0, C12:1cis9, C13:0, C15:0, and C17:0) on Farm A. In particular, as previously supposed, cattle in Farm A, were fed a potentially acidogenic diet characterized by a high NFC content and a low NDF and peNDF >8 mm. Moreover, some odd-chain FA, like C15:0 and C17:0, appear to show a specific peNDF related trend. This has also been verified, even though only numerically, for other markers of acidogenic diet, such as C7:0, C9:0, C10:0, C11:0, C12:0, and C13:0.

It appears that cattle receiving the same diet showed different values of milk fat and fatty acids profiles throughout lactation, particularly in early lactation. These differences in milk fat levels and fatty acid profile within farms might be induced by several factors, including different body conditions during the dry period or different energy status and partitioning in transition and early lactation, different feed intakes, and different feeding behavior (Holtel et al., 1990; Dewhurst et al., 2000). The MFD cattle seems to have a similar milk FA profile of cattle fed acidogenic diets. Therefore the origin MFD in the studied commercial dairy herds could be related to a change of the metabolic status towards an acidotic condition.

Conclusions

The results of the present work demonstrated that fresh cattle showing MFD in commercial dairy herds are characterized by an higher milk yield but tendentially lower mammary energy output or ECM production in early and mid-lactation, and by a different fatty acid profile from cattle having physiological levels of fat in milk. Thus, the occurrence of MFD in fresh dairy cattle could be considered as an index of a potentially lower efficiency of diet conversion connected to metabolic anomalies. Further studies considering TMR and feed bunk management, cow feeding behavior and farm management, such as possible differences in dry matter intake between cows groups are needed to obtain a better understanding of the reasons of milk fat and FA profiles heterogeneity within farm.

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