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The influence of dietary nitrogen reduction and conjugated linoleic acid supply to dairy cows on fatty acids in milk and their transfer to ripened cheese

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ABSTRACT

The aim of this study was to investigate the consequences of reducing the dietary crude protein content, with or without a supply of protected conjugated linoleic acid (CLA), on the milk fatty acid (FA) yield and recovery in 90 d ripened cheese. Twenty mid-lactation Friesian dairy cows were reared for 4 periods of 3 wk each in groups of 5, following a 4 × 4 Latin square design. Cows were fed 4 different rations, consisting of a combination of the 2 dietary crude protein levels [150 (CP15) or 123 (CP12) g of crude protein/kg of dry matter], with or without a conjugated linoleic acid supply (80 g/d, providing 5.57 and 5.40 g/d of C18:2 *cis*-9,*trans*-11 and C18:2 *trans*-10,*cis*-12, respectively). Milk yield was recorded. Twice in each period, milk samples were analyzed for protein, fat, and lactose content, and 10 L milk samples (pooled by group) were processed to produce 96 cheeses, which were ripened for 90 d. Milk and cheese fat were analyzed for their FA profiles. Milk and cheese FA were expressed as daily yields and relative proportions, and nutrient recoveries were computed. Dietary crude protein reduction had small or no effects on the yield and relative presence of FA in milk and cheese, except for a small increase in mid-chain branched saturated fatty acids. The CLA supply strongly reduced the yield of various categories of FA, and had major effects on short-chain FA of de novo synthesis, leading to changes in the relative proportions of the various FA in milk and cheese. The addition of CLA tended to reduce uniformly the recovery of all milk constituents and of short-, medium-, and long-chain FA groups, but we observed large differences among individual FA with apparent recoveries ranging between 640 and 1,710 g/kg. The highest recoveries were found for polyunsaturated long-chain FA, the lowest for saturated or monounsaturated short- or medium-chain FA. A notable rearrangement of these FA components, particularly the minor ones, took place during ripening.
Key words: conjugated linoleic acid, dietary protein, fatty acid, milk, cheese

INTRODUCTION

Reducing the environmental impact of animal farming and producing milk and cheese with improved nutritional characteristics are among the most important challenges for our dairy industry today. Feeding dairy cows excess protein contributes to environmental N pollution and could lead to unnecessary feeding expenditure due to the high costs of protein sources (Kebreab et al., 2002), but the use of low-protein rations as a means of reducing N loss in the environment could cause changes in the nutritional and technological characteristics of milk. Cesaro and Schiavon (2015) showed that CP restriction influenced the coagulation, curd firmness, and syneresis of Holstein-Friesian milk. Leonardi et al. (2003) and Cabrita et al. (2007) reported that a reduction in dietary CP content caused some alteration to the milk fatty acid (**FA**) profile: a decrease in the proportion of medium-chain FA (C16:0), and an increase in C18:1 *trans* isomers and some CLA isomers. An increase in the milk fat content of these *trans*-octadecenoic isomers and related metabolites, such as CLA isomers, has been frequently associated with milk fat depression (Griinari et al., 1998; Bauman et al., 2008; Shingfield et al., 2010). The use of a commercial rumen-protected CLA mixture has also consistently been found to considerably reduce the milk FA yield and profile (Perfield II et al., 2002). Because dietary CP reduction has been related to an increase in these bioactive milk FA (Cabrita et al., 2007), it is possible that dietary protein and CLA supplementation interact with respect to milk FA composition and FA yield.

The proportion of milk processed into cheese is growing worldwide (IDF, 2015), and there is interest in assessing the effects of different feeding treatments on

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cheese quality (Colombari et al., 2001; Angulo et al., 2012) including the FA profile of milk and the derived cheese and the pathways of FA transfer from milk to cheese. Some authors have suggested that the FA profile of fresh cheese reflects that of the milk from which it has been made (Allred et al., 2006; Bodkowski et al., 2016); others (Mordenti et al., 2015; Bocquel et al., 2016) have suggested that alterations to the FA profile probably occur during ripening. However, few studies have specifically addressed the transfer of individual FA from milk to cheese (Cattani et al., 2014).

The aim of this work was to study the effect of conventional or low-protein diets, with or without CLA supplement, on milk and cheese individual FA yields and proportions, and to investigate the effect of cheese manufacturing and ripening on individual FA recoveries in cheese.

MATERIALS AND METHODS

All experimental procedures involving animals were approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padua.

Animals, Diets, Milk Yield, and Composition

A detailed description of the animal characteristics, diets, experimental design, production performance, and N balance is given in Schiavon et al. (2015). Briefly, 20 Holstein-Friesian cows with equal milk yield (31.0 ± 1.4 kg/d), DIM (174 ± 6 d), parity (2.0 ± 0.36), BW (641 ± 26 kg), and BCS (2.9 ± 0.07) were randomly allotted to groups of 5 animals in 4 pens. The cows were fed once a day on TMR according to a 4 × 4 Latin square design, over 4 periods of 3 wk (2 for adaptation and 1 for sample collection) and 4 dietary treatments. The sequence of treatments was designed to ensure that each group received rations with low (**CP12**) or control CP (**CP15**) contents for 6 consecutive weeks, the rumen-protected CLA was added or withdrawn without a simultaneous change in the CP content of the diet, and the change in CP was made without a simultaneous change in CLA status, as illustrated in Schiavon et al. (2015). Control rations were formulated following NRC (2001) recommendations to meet the energy and nutrient requirements for 30.0 kg/d of milk yield with 35, 34, and 47 g/kg of protein, fat, and lactose, respectively, with a predicted DMI of 21 kg/d. The 2 CP12 rations were formulated from the ingredient composition of the control rations by replacing soybean meal with barley grain (Table 1). The CP15 and the CP12 rations contained 150 and 123 g/kg DM of CP, respectively. The $CP15_{CLA}$ and $CP12_{CLA}$ rations were supplemented with 80 g/d of a top-dressed commercial lipid-coated CLA mixture (Sila, Noale, Italy), as described in Schiavon et al. (2011), to provide about 5.57 and 5.40 g/d of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA isomers, respectively.

Cows were milked twice a day, at 5:00 a.m. and 5:30 p.m., and individual milk yield was recorded at each milking using an automatic milking system for the herringbone parlor coupled with automatic recording software (Alpro; DeLaval, Tumba, Sweden). During the third week of each period, individual milk samples from the morning and evening milkings were analyzed each day for fat, protein, and lactose content using the International Dairy Federation (IDF) procedure (IDF, 2000) and a MilkoScan apparatus (Foss Electric, Hillerød, Denmark). The average computed morning and evening milk fat yields and milk protein yields were very similar: 0.473 ± 0.153 and 0.481 ± 0.174 kg/d for fat, and 0.495 ± 0.113 and 0.463 ± 0.102 kg/d for protein, respectively, in agreement with our previous observations on the same herd of Holstein-Friesian cows fed corn-silage-based TMR with milking intervals of 12 h. We assumed no differences in FA profile between the milk collected in the morning and evening milkings (Larsen et al., 2012).

On d 2 and 4 of the last week of each experimental period, individual milk samples (2,100 mL) from the morning milking were collected for analysis, and 2 cheesemaking sessions were held. The milk samples from each cow were pooled by group and poured into 2 laboratory cheese vats (11 L capacity; Pierre Guerin Technologies, Mauze, France). Two aliquots of 50 mL were sampled from each vat. One aliquot from each was immediately analyzed for fat, protein, and total solids using the MilkoScan apparatus (IDF, 2000); the other was stored at −80°C and later analyzed for milk FA profile using 2-dimensional gas chromatography (GC × GC), according to Pellattiero et al. (2015a).

Cheesemaking and Cheese Composition

Milk was processed into cheese without preliminary heat or homogenization treatments, and cheese manufacturing was carried out simultaneously for the 4 experimental treatments during each cheesemaking session (2 sessions per experimental period, 8 in total, for a grand total of 32 cheesemakings). Cheeses were made according to the same procedure as described in previous studies (Cattani et al., 2014). During each cheesemaking, 3 cheese wheels were produced per vat, for a total of 96 wheels (4 groups × 4 periods × 2 sessions × 3 wheels). All wheels were ripened for 90 d in a cell at 15°C and 85% relative humidity and weighed at regular intervals from d 1 to 90 of ripening (around 200