



Effect of phenological stage and proportion of fresh herbage in cow diets on milk fatty acid composition

M. Coppa^{a,*}, A. Ferlay^{b,c}, G. Borreani^a, A. Revello-Chion^d, E. Tabacco^a, G. Tornambé^{b,c}, P. Pradel^e, B. Martin^{b,c}

^a University of Turin, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, 10095 Grugliasco (Turin), Italy

^b INRA, UMR 1213 Herbivores, F-63122 Saint-Genès-Champagnelle, France

^c Clermont Université, VetAgro Sup, UMR Herbivores, BP 10448, F-63000 Clermont-Ferrand, France

^d Associazione Regionale Allevatori del Piemonte, Via Livorno 60, 10144 Turin, Italy

^e UE1296 Monts-d'Auvergne, INRA, F-63210 Orcival, France

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ABSTRACT

The aim of this work was to test the effect of herbage phenology on the fatty acid (FA) composition of milk and its interaction with fresh herbage proportion in cow diets in controlled conditions, as well as to validate the results in on-farm conditions. Three experiments were conducted in controlled conditions, and were designed to be representative of dairy systems with increasing fresh herbage proportions in cow diets: low (25% of diet dry matter (DM)); high (75% of diet DM), and full grazing (100% of diet DM). The fourth experiment was a survey conducted in commercial farms that adopt feeding systems similar to those of controlled conditions. An overall effect of herbage phenology on milk FA composition was observed. The number of FA that were affected by herbage phenology was higher as the proportion of fresh herbage in the cow diets increased. The C16:0, total even chain saturated FA, odd chain FA, branched-chain FA, C18:1c9, and monounsaturated FA concentrations in milk increased with increasing herbage phenology, whereas the *de novo* synthesized FA, C18:1t11, CLAc9t11, C18:3n-3 and total polyunsaturated FA concentrations decreased. When low proportions of fresh mature herbage was fed to cows, the FA profile of the derived milk differed from the control diet (without fresh herbage) only for the higher concentrations of C18:1t11, CLAc9t11, and C18:3n-3, whereas the differences were greater and significant for several FA for similar proportions of fresh herbage at an early phenological stage. The results obtained in controlled conditions were confirmed on-farm: the C18:1t11 and CLAc9t11 were the most sensitive FA to the effect of fresh herbage phenology. However, when low fresh herbage proportion were used in cow diets, the effect of herbage phenology on milk FA concentration was negligible.

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Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber; BCFA, sum of branched-chain FA; CLA, conjugate linoleic acid; CP, crude protein; *de novo* FA, sum of *de novo* synthesized FA; ECSFA, sum of even-chain saturated FA; FA, fatty acid; DM, dry matter; MUFA, sum of monounsaturated FA; OCFA, sum of odd-chain FA; OMD, organic matter digestibility; PUFA, sum of polyunsaturated FA; SFA, saturated FA; TMR, total mixed ration.

* Corresponding author.

E-mail address: mauro.coppa@unito.it (M. Coppa).

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1. Introduction

In Europe, grasslands occupy about 39% of the utilized agricultural area, and grazing dairy cows account for about 24 million grazing livestock units (Peeters, 2012). Thus, pasture-based dairy farming systems play a key role in European milk production. These systems are also interesting because of the high nutritional value of the produced milk, in particular referring to the fatty acid (FA) composition. Feeding cows with fresh herbage increases milk polyunsaturated FA (PUFA), conjugated linoleic acids (CLA) and $n-3$ FA and decreases saturated FA (SFA) and $n-6$ FA concentrations in milk (Shingfield et al., 2013; Chilliard et al., 2007; Dewhurst et al., 2006), and thus a more favorable FA profile is reached for human health (Kratz et al., 2013; Givens, 2010). Recently, some dairy companies, in various EU countries (i.e. France, Belgium, The Netherlands), have applied a price premium for cow milk rich in FA with a putative positive effect on health (i.e. high concentrations of $n-3$ and PUFA, and low concentrations of SFA) (Borreani et al., 2013). However, the FA composition of pasture-derived milk and dairy products varies to a great extent according to herbage characteristics and diet formulation (i.e. forage-to-concentrate ratio) (Chilliard et al., 2007; Dewhurst et al., 2006), whereas animal related factors (i.e. breed and lactation stage) have been shown to have negligible effects (Coppa et al., 2013; Ferlay et al., 2006). The relationships between the botanical composition of a pasture and FA concentrations of milk have been studied in depth, both in controlled conditions (Coppa et al., 2011; Ferlay et al., 2006) and in on-farm conditions (Revello-Chion et al., 2010; Bugaud et al., 2002; Collomb et al., 2002). However, some factors other than botanical composition, such as grazing management, and animal characteristics may affect milk FA profile (Coppa et al., 2015; Couvreur et al., 2006). Herbage phenological stage can also play an important role, as variations in the FA concentration of herbage have been reported to vary accordingly by several authors (Glasser et al., 2013; Buccioni et al., 2012; Khan et al., 2012). The effects of herbage phenology on milk yield and FA composition have been studied in sheep grazing on different Mediterranean species of forages or their mixtures (reviewed by Cabiddu et al., 2005). These authors have shown an increasing trend of C18:3 $n-3$ and rumenic acid (CLAc9t11) with herbage maturity. On the contrary, Vanhatalo et al. (2007) have shown a progressive decrease in both these FA and PUFA concentration in milk with increasing herbage maturity, when cows were fed ensiled temporary grasslands at different phenological stages. Similar results have recently been found by Kälber et al. (2014) for C18:3 $n-3$, but not for CLAc9t11, when cows were fed indoors with fresh herbage at vegetative and reproductive stages. However, little is known about how fresh herbage phenology interacts with various proportions of fresh herbage in cow diets on milk FA concentrations.

The work was aimed at evaluating the effect of herbage phenological stage, and its interaction with different fresh herbage proportion in cow diet (corresponding to increasing intensification of production conditions), on FA composition of milk in controlled conditions, and at verifying the results on-farm.

2. Materials and methods

2.1. Animals and feeding treatments

Three experiment with increasing proportion of fresh herbage in cow diet have been conducted in controlled conditions (Experiments 1, 2 and 3) and a farm survey has been carried out on commercial dairy farms with feeding systems similar to those studied in the controlled conditions (Experiment 4). Experiments 1 and 2 were carried out at the INRA experimental farm of Marcenat in an upland area in central France (45°15' N, 2°55' E; altitude 1135–1215 m; annual rainfall 1100 mm), while Experiment 3 was conducted on a commercial farm located in Scarnafigi (CN), in the Piedmont lowlands, in North-West Italy (44°41' N, 7°34' E, altitude 295 m; annual rainfall 730 mm). The farms involved in Experiment 4 were distributed throughout the Torino and Cuneo Provinces in Piedmont, in North-West Italy, as described hereafter.

2.1.1. Experiment 1: Full grazing

After a two-week pre-experimental period, during which 18 winter-calving Montbéliarde cows grazed on the same pasture, cows were divided into three equivalent groups of six, considering body weight (612 ± 9.1 kg), milk yield (18.0 ± 0.4 kg/cow \times day), milk fat and protein contents (37.5 ± 0.43 , and 31.9 ± 0.22 g/kg of milk, respectively), days in milk (177 ± 7.0), and parity. The three groups were assigned to three pasture plots, differing for floristic diversity, in order to understand the effect of the evolution of herbage phenology on different pasture types: a temporary grassland; a moderately biodiversified permanent grassland; and a highly biodiversified permanent pasture. Cows did not receive any concentrate supplementation. During the 3-week experiment on the first growth cycle, cows grazed during day and night by strip grazing (electric fences were moved forward every two days). Individual milk samples were collected once a week at weeks 1 and 3, which corresponded to the early (stem elongation) and late (late flowering) herbage phenological stages.

2.1.2. Experiment 2: Intermediate fresh herbage proportion

A group of 5 Montbéliarde winter-calving dairy cows (594 ± 14.1 kg of body weight; 15.1 ± 1.5 kg/cow \times day of milk yield; 109 ± 11.9 days in milk) strip-grazed a botanically diversified pasture, for four consecutive weeks in the first growth cycle. Cows were supplemented with 3.8 kg/cow \times day of a commercial concentrate (on a dry matter (DM) basis, wheat, 37.3%, bran, 22.6%, wheat gluten, 14.4%, sunflower cake, 5.4%, rapeseed cake, 4.6%, cereal by-products, 10.0%, molasses, 2.5%, and carbonate, minerals and vitamins, 3.2%), and fresh herbage represented about 75% of the dietary DM. Individual milk samples

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