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The use of 2-dimensional gas chromatography to investigate the effect of rumen-protected conjugated linoleic acid, breed, and lactation stage on the fatty acid profile of sheep milk

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ABSTRACT

In this study, 2-dimensional gas chromatography (GC × GC) was used to obtain a detailed fatty acid (FA) profile of sheep milk and to evaluate the effects of a rumen-protected conjugated linoleic acid (rpCLA) supply, breed, days in milk (DIM), sampling period, and number of lambs suckling on the FA profile. Twenty-four ewes, from 3 autochthonous breeds of the Veneto Alps (Brogna, Foza, and Lamon), were housed in 6 pens (2 pens/breed), according to DIM (38 ± 23 d) and body weight (61 ± 13 kg). The ewes and their offspring of 3 pens (1 pen/breed) were fed ad libitum a total mixed ration (control), and the other animals received the same diet supplemented with 12 g/d per ewe, plus 4 g/d for each lamb older than 30 d, of an rpCLA mixture. The study lasted 63 d. Two composite milk samples for each ewe were prepared during the first and second months of the trial. The pooled milk samples were analyzed in duplicate for FA profile by 2-dimensional gas chromatography, which allowed us to obtain a detailed FA profile of sheep milk, with 170 different FA detected, including many that were present in small concentrations. The milk relative proportions of individual FA, groups of FA, or FA indices were analyzed by PROC MIXED of SAS (SAS Institute Inc., Cary, NC), considering diet, breed, DIM, and sampling period as sources of variation. The random effect of animal was used to test diet, breed, and DIM, whereas the effects of period were tested on the residual. Breed had a small influence on milk FA profile, mainly on branched- and odd-chain FA. Within breed, animal repeatability for the relative proportions of milk FA was notable for almost all monounsaturated FA and for saturated FA with 14 to 19 carbon atoms, except C16:0, and less so for polyunsaturated FA. The inclusion of rpCLA (CLA *cis*-9,*trans*-11 and CLA *trans*-10,*cis*-12) increased the presence of the same CLA isomers in the milk as well as that of CLA *trans*-9,*trans*-11, and decreased the proportions of de novo-synthesized short-chain FA. From a cluster analysis based on the matrix of correlation coefficients among all FA relative proportions, 3 main FA groups were observed: the first included mainly odd- or branched-chain saturated FA, C18:0, C16:0 and CLA *trans*-10,*cis*-12; the second included monounsaturated FA or polyunsaturated FA with 16 to 20 carbons, CLA *cis*-9,*trans*-11, and CLA *trans*-9,*trans*-11; and the third included short- to medium-chain saturated FA, polyunsaturated FA with 2 to 5 double bonds, and 3 CLA isomers not affected by rpCLA addition (CLA *trans*-11,*cis*-13, CLA *cis*-9,*cis*-11, and CLA *cis*-10,*cis*-12).
Key words: conjugated linoleic acid (CLA), ovine milk, fatty acid, sheep breed, 2-dimensional gas chromatography

INTRODUCTION

Compared with bovine products, ovine products are known for their greater content of some FA that are considered beneficial for human health (Wahle et al., 2004; Sinclair, 2007), such as PUFA including linolenic acid, n-3 PUFA, and isomers of CLA (Dilzer and Park, 2012; Shingfield et al., 2013). Sheep milk is seldom consumed as a fresh product, so these effects would be exploited by the consumption of processed milk (Prandini et al., 2007), especially cheese (Nudda et al., 2005; Buccioni et al., 2010). In the case of bovine milk, the recovery of the majority of PUFA and CLA from milk to ripened cheese is thought to be greater than 80% (Cattani et al., 2014).

The FA profile of ovine milk depends partly on the genetic and physiological characteristics of the ovine breeds but also from feeding system (Pulina et al., 2006; De La Fuente et al., 2009). Milk obtained from animals kept on grass-based diets is commonly richer in these FA compared with that obtained from animals kept on hay-, silage- or concentrate-based diets (Jutzeler van Wijlen and Colombani, 2010). The increased use indoor of corn silage and concentrates and the abandonment

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of seasonality in favor of continuous production systems are factors that would impair the quality of the lipid component of sheep milk. Nevertheless, the use of some rumen-protected fat can be a useful tool to deliver beneficial FA in tissues and milk. The use of rumen-protected CLA (**rpCLA**) supplements, containing the 2 most promising CLA isomers, C18:2 *cis*-(***c***)9,*trans*-(***t***)11 and C18:2 *t*10,*c*12, has been found to be effective in modifying the FA profile of both beef meat (Schiavon et al., 2011) and cow's milk (Pappritz et al., 2011). Less is known about influencing the FA profile of sheep milk (Weerasinghe et al., 2012) and lamb (Mir et al., 2000; Terré et al., 2011) tissues through supplementation of CLA on indoor diets based on corn silage and concentrate-based diets. We hypothesize that administration of rpCLA could be an effective means to increase the CLA content of milk sheep, and a way to modify the presence of other FA because of possible interferences with the lipid metabolism.

The availability of powerful analytical methodology and equipment, such as 2-dimensional gas-chromatography (**GC × GC**), makes a detailed assessment of the FA profile feasible (Manzano et al., 2011), improving our ability to study the influence of different source of variations, even for molecules present in small amounts but that could exert important biochemical functions. A basic characteristic of the GC × GC technique is the 2-dimensional ordered structure of the chromatogram, which makes identification of compounds more reliable than in traditional GC (Adahchour et al., 2008). This technique would be useful for the analysis of samples where compounds are present in very different concentrations across a wide range of different FA (Vlaeminck et al., 2007).

The aim of this research was to study the effects of diet supplementation with a rumen-protected mixture of 2 CLA isomers (CLA *c*9,*t*11, CLA *t*10,*c*12) on a detailed FA profile. In particular, our objective was to quantify the increase of these 2 CLA isomers in milk fat, to analyze possible effects on other CLA isomers, and to test the hypothesis that CLA addition can modify lipid metabolism and particularly the de novo synthesis of FA in the mammary gland. An additional objective was to study the effect of animal source of variation of the detailed FA profile of milk collected from ewes of 3 autochthonous breeds (Brogna, Foza, and Lamon) of the Veneto Alps, using the GC × GC technique.

MATERIALS AND METHODS

Animals, Feeding, and Milk Sampling

This experiment was carried out at the "Lucio Toniolo" Experimental Farm of the University of Padova in Legnaro (Padova, Italy) on animals undergoing an in situ conservation program of the sheep breeds autochthonous of the Alpine areas of the Veneto region (northeast Italy). Animals were treated according to the *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1988).

Twenty-four ewes with their 31 suckling lambs, of Brogna (10 ewes), Foza (9 ewes), and Lamon (5 ewes) breeds, were allotted in 6 pens (3 × 6 m each) of an open barn (2 pens/breed), homogeneous for DIM (38 ± 23 d) and ewe BW (61 ± 13 kg). The 12 ewes of 3 pens (1 pen/breed) were fed a control diet, and the 12 ewes of the other 3 pens received the same diet top dressed and mixed with 12 g/d per ewe, plus 4 g/d for each lamb older than 30 d, of a commercial rpCLA supplement (SILA, Noale, Italy) that contained, as detailed in Schiavon et al. (2010), 79.2 and 76.8 g/kg of C18:2 *c*9,*t*11 and C18:2 *t*10,*c*12 isomers, respectively.

The experimental conditions and the composition of diets were described in detail by Bittante et al. (2014). Briefly, the control diet was composed of 37.3, 26.0, 11.1, 11.0, 6.4, 6.6, and 1.6% DM corn grain, corn silage, dried sugar beet pulp, soybean meal, wheat bran, wheat straw, and a vitamin mineral mixture, respectively. From chemical analysis performed on each feed ingredient and from ingredient compositions, the diet contained 13.0, 29.3, 14.6, and 34.7% DM of CP, NDF, ADF, and starch, respectively, and 11.4 MJ/kg of DM of ME (Bittante et al., 2014). Diet ingredients were mixed with water to reach an average dietary DM content of 50.4% and offered ad libitum as TMR. The amount of each feed ingredient loaded into the mixer wagon, and the weight of the mixture uploaded in the manger of each pen was recorded daily. The orts remaining in the mangers were weighed weekly by pen. The study lasted 63 d. At the beginning and end of the trial and every 2 wk, the ewes and their offspring were individually weighed and scored for BCS by a single trained technician.

Six times during the experiment, in the morning, the ewes were separated from their offspring for 2 h, and milk samples were collected from each ewe, refrigerated, and analyzed for fat, protein, and lactose contents using a MilkoScan FT2 (Foss, Hillerød, Denmark) according to Bittante et al. (2014). For the purposes of current work, an aliquot of each milk sample was conserved at −80°C. Prior to analysis, milk samples were thawed and pooled in 2 composite samples for each ewe: the first pooled the 3 milk samples collected during the first month of the trial (period A) and the second pooled the 3 samples collected during the second month of the trial (period B).