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Milk phospholipids: Organic milk and milk rich in conjugated linoleic acid compared with conventional milk

T. Ferreiro, L. Gayoso, and J. L. Rodríguez-Otero[1]
Instituto de Investigación y Análisis de Alimentos, Universidad de Santiago de Compostela, Facultad de Veterinaria, 27002 Lugo, Spain

ABSTRACT

The objective of this study was to compare the phospholipid content of conventional milk with that of organic milk and milk rich in conjugated linoleic acid (CLA). The membrane enclosing the fat globules of milk is composed, in part, of phospholipids, which have properties of interest for the development of so-called functional foods and technologically novel ingredients. They include phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylserine (PS), and the sphingophospholipid sphingomyelin (SM). Milk from organically managed cows contains higher levels of vitamins, antioxidants, and unsaturated fatty acids than conventionally produced milk, but we know of no study with analogous comparisons of major phospholipid contents. In addition, the use of polyunsaturated-lipid-rich feed supplement (extruded linseed) has been reported to increase the phospholipid content of milk. Because supplementation with linseed and increased unsaturated fatty acid content are the main dietary modifications used for production of CLA-rich milk, we investigated whether these modifications would lead to this milk having higher phospholipid content. We used HPLC with evaporative light scattering detection to determine PE, PI, PC, PS, and SM contents in 16 samples of organic milk and 8 samples of CLA-rich milk, in each case together with matching reference samples of conventionally produced milk taken on the same days and in the same geographical areas as the organic and CLA-rich samples. Compared with conventional milk and milk fat, organic milk and milk fat had significantly higher levels of all the phospholipids studied. This is attributable to the differences between the 2 systems of milk production, among which the most influential are probably differences in diet and physical exercise. The CLA-rich milk fat had significantly higher levels of PI, PS, and PC than conventional milk fat, which is also attributed to dietary differences: rations for CLA-rich milk production included linseed supplement and contained less maize meal than conventional rations and a greater proportion of unsaturated fatty acids and salts. The relative proportions of the phospholipids studied were similar in all 3 types of milk, descending in the order PE > (PC, SM) > PS > PI, with PC being slightly more abundant than SM in organic milk and vice versa in CLA-rich milk.

Key words: phospholipids, organic milk, conjugated linoleic acid-rich milk, conventional milk

INTRODUCTION

Phospholipids (glycerophospholipids and sphingophospholipids) are among the main structural components of many biological membranes, including the membranes delimiting the fat globules of milk. They are amphiphilic molecules with a hydrophilic head group and a hydrophobic tail. Glycerophospholipids are diglycerides linked via the third glycerol hydroxyl to a phosphate group that may, in turn, bear a further organic group, usually a small group such as choline, ethanolamine, serine, inositol, or glycerol. In sphingophospholipids, a phosphate group with a small organic moiety is esterified to the terminal sphingosine hydroxyl of a ceramide.

Over the past 20 yr or so, much research has been conducted on the nonstructural activities of phospholipids, the nutritional implications of these activities, and the technological aspects of their possible use in so-called functional foods (Contarini and Povolo, 2013). Phospholipids have been associated with inhibition of colorectal cancer (Dillehay et al., 1994; Lemonnier et al., 2003), reduction of blood cholesterol levels (Noh and Koo, 2004; Duivenvoorden et al., 2006), enhancement of brain function (Pepeu et al., 1996), prevention of infection (Vesper et al., 1999), and protection of the gastric mucosa (Kivinen et al., 1992).

Organic agricultural production is "an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural

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[1]Corresponding author: jlr.otero@usc.es

substances and processes" (European Council, 2007). For a food product to be certified as organic, it must satisfy a comprehensive series of requisites on aspects of production that, in the case of milk, range from livestock management and feed to the labeling of the final product. Recent years have seen increased demand for organic foods, including milk and other dairy products. According to several studies, milk from organically managed cows contains higher levels of vitamins, antioxidants, and unsaturated fatty acids than conventionally produced milk (Bergamo et al., 2003; Ellis et al., 2006). However, we know of no study with analogous comparisons of major phospholipid contents. The aim of the first part of our study was to fill this gap.

Conjugated linoleic acid comprises all double-bond-location isomers of linoleic acid in which the 2 C=C bonds are conjugated. The most abundant CLA isomer in cow milk is rumenic acid (*cis*-9,*trans*-11 18:2). The concentration of CLA in milk fat has been reported to range from 0.5% in the milk of cows fed grass silage to 2.1% in that of grazing cows (Mohammed et al., 2009). Similarly, in the region in which the present study was carried out, Roca-Fernández et al. (2012) found CLA levels of 1.14% in the milk fat of grazing cows compared with 0.49% in milk fat of silage-fed cows, the difference being greatest when fresh grass was in greatest supply. In trials evaluating this preponderant influence of diet on milk CLA concentration, increasing green grass consumption or supplementing rations with vegetable oil linoleic acid has brought about a rapid increase in the CLA content of milk fat (Chichlowski et al., 2005; Huth et al., 2006). Importantly, therefore, these increases in CLA content are achieved naturally, by modifying the diet, without supplementing the milk with any kind of chemical additive.

The use of polyunsaturated-lipid-rich feed supplement (extruded linseed) has also been reported to increase the phospholipid content of milk from cows fed maize silage (López et al., 2008). Because supplementation with linseed and increased unsaturated fatty acid content are the main dietary modifications used for production of CLA-rich milk, in the second part of this study we investigated whether these modifications likewise lead to this milk having higher phospholipid content.

MATERIALS AND METHODS

Samples

For both the study of organic milk and the study of CLA-rich milk, a sample of conventionally produced milk was taken on the same day in the same area. Organic milk samples comprised 16 pairs of cow milk samples supplied by Leche de Galicia S.L. (Vilalba, Galicia, Spain). They were collected at regular intervals between January 2012 and March 2013. Each pair comprised a sample of organic milk and a sample of conventional milk, both taken on the same day. Both samples were taken following discharge of milk from farms in Galicia (northwest Spain) into ~40,000-L tanks in the case of organic milk (the entire 2-d production of the area) or ~100,000-L tanks in the case of conventional milk (part of the 2-d production of the area). The samples, therefore, can be considered representative averages for the catchment area of the processing plant. All samples arrived chilled and, when necessary, were frozen pending extraction of polar lipids. The CLA-rich milk samples comprised 8 pairs of raw cow milk samples supplied by Cooperativa Feiraco (Negreira, Galicia, Spain). They were collected at regular intervals between July 2012 and March 2013. Each pair comprised a sample of conventionally produced milk and a sample of CLA-rich milk with approximately 1.8 mg of CLA per 100 mg of milk fat, both taken on the same day. The CLA-rich samples were taken from 25,000-L tanks containing the total production of 2 d, and conventionally produced samples were taken from 100,000-L tanks containing part of the production of 2 d; thus these samples, too, can be considered representative averages of the catchment area of the processing plant. All samples arrived chilled and, when necessary, were frozen pending extraction of phospholipids.

Fat Content

Fat content was determined in accordance with International Dairy Federation Standard 141C (IDF, 2000) by mid-infrared spectroscopy on a Milkoscan FT2 apparatus (Foss, Hillerød, Denmark). All samples were analyzed in duplicate.

Analysis of Phospholipids

The phospholipid fraction of the milk was considered to consist entirely of phosphatidylethanolamine (**PE**), phosphatidylinositol (**PI**), phosphatidylcholine (**PC**), phosphatidylserine (**PS**), and sphingomyelin (**SM**). Phospholipids were extracted, and contents PE, PI, PC, PS, and SM were determined, using slightly modified versions of the methods of Rombaut et al. (2005). Briefly, phospholipids were extracted into chloroform:methanol and the extracts were stored at −42°C in amber vials pending analysis by HPLC. All samples were extracted in duplicate. The HPLC apparatus (Shimadzu, Kyoto, Japan), comprising a degasser, a solvent delivery module, a controller module, a column oven, a Rheodyne manual injector valve and