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Milk fat globule size, phospholipid contents and composition of milk from purebred and Alpine-crossbred Mid-Eastern goats under confinement or grazing condition

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ABSTRACT

Milk fat globule (MFG) size and phospholipids (PL) content and composition were determined in milk collected at 65 (pretreatment), 110, 135 and 170 days of lactation from goats randomly assigned to grazing in Mediterranean brushland or fed clover hay indoors, in addition to concentrate. Daily feed intake and dietary contents of neutral detergent fibre and acid detergent fibre were higher in grazing goats, associated with milk richer in fat, with larger MFGs and 20% higher PL content. Smaller MFGs, produced by all confinement groups, was associated with 15 μg g^{-1} fat higher milk PL content. The greatest effect was found in the Damascus goats, with over 44% higher PL concentration, on milk fat basis, in the confined compared with grazing group. Our understanding of how PL content is modulated by the interaction between genetic background and nutrition will enable to achieve either PL-rich milk or PL-enriched milk fat.

1. Introduction

Milk fat is secreted in a unique structure termed the milk fat globule (MFG) that consists of a triglyceride (TG) core covered with three layers of phospholipids (PLs), termed the MFG membrane. The MFG membrane consists of PLs, sphingolipids, glycolipids, cholesterol and proteins, all derived from the endoplasmic reticulum and apical membrane of the mammary epithelium (Mather & Keenan, 1998). The MFGs are secreted in a wide range of sizes, from the nanometre scale to over 15 μm in human milk (Michalski, Briard, Michel, Tasson, & Poulain, 2005) and in bovine milk (Mulder & Walstra, 1974), whereas a smaller MFG within the size range of 0.53–8.58 μm is found in caprine milk (Attaie & Richter, 1999). Understanding how MFG size is regulated is important for both industrial and health-related reasons. MFG size affects cheese ripening and structure, as well as stability of dairy products (Lopez et al., 2011; Michalski et al., 2003). Moreover, MFG size is tightly associated with its lipid composition, with a greater content of PL and sphingolipids in small versus large globules (Lopez et al., 2008; Mesilati-Stahy, Mida, & Argov-Argaman, 2011).

A higher PL content in the human diet is desirable as PLs are considered to be health-promoting food ingredients (Kullenberg, Taylor, Schnieder, & Massing, 2012). For example, the plasma lipid profile was shown to be improved by the consumption of a PL-rich diet (Burgess et al., 2005). Furthermore, reduced occurrence of colon cancer was demonstrated when sphingomyelin (SM) was supplemented to a colon cancer-induced murine model (Dillehay, Webb, Schmelz, & Merrill, 1994). In another study, fortification of a milk replacement with MFG membrane decreased the number of days with fever and ear infections and improved cognitive performance of babies by the age of 1 (Lonnerdal, Timby, Domellof, Domelof, & Hernell, 2014; Timby, Domellif, Lonnerdal, & Domellof, 2014). Therefore, understanding how to increase the content of PL in milk would situate milk as a health-promoting food and modify the health implications of milk consumption dramatically.

MFG size and consequently PL and sphingolipid content in milk were found to be associated with various hormonal, metabolic, and genetic factors. However, the mechanism controlling MFG size is still unclear. Insulin and energy balance (Argov-Argaman, Mbogori,

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Sebastian, Shamay, & Mabjeesh, 2012), lactation stage (Bitman & Wood, 1990), progesterone and stage of the estrous cycle (Mesilati-Stahy, Malka, & Argov-Argaman, 2015) have been suggested to participate in the regulation of MFG size. A possible explanation was the partitioning of unsaturated fatty acids between the mammary gland and peripheral tissues. Lower total milk fat content and smaller MFG were found when cows were fed a higher energy content diet compared with a high forage diet (Argov-Argaman, Mesilati-Stahy, Magen, & Moallem, 2014). Therefore, it seems that MFG size is affected by the dietary content of both energy and neutral detergent fibre (NDF). Besides dietary and hormonal regulation, there is a genetic component to MFG size as well (Argov-Argaman, Mida, Cohen, Visker, & Hettinga, 2013). In the bovine, a genetic predisposition for production of large versus small MFGs has been shown to interact with the dietary effect on MFG size (Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007). However, knowledge of MFG size in small ruminants is still scarce; nonetheless, goat MFG are known to be smaller than those of bovine and therefore offer a richer source of PL and sphingolipids in the human diet. In addition, smaller MFGs result in softer curd during the cheese-making process, which may be associated with better digestibility (Park, 2009). A comparison of bovine, human and goat milk PL compositions showed slight differences, with higher SM in goat milk than in cow milk, and slightly lower phosphatidylcholine (PC) (Renner, Schaafsma, & Scott, 1989), which are two of the major polar lipids in milk (Mele, Buccioni, Serra, Antongiovanni, & Secchiari, 2008). However, to the best of our knowledge, the breed effect on milk PL content and composition has never been studied in dairy goats.

A variety of dairy goat breeds are used in Israel, with the local Mamber (also termed Baladi, Syrian mountain) and Damascus (also termed Shami) goats raised under grazing, and Alpine or Saanen goats under confined management (Landau, Perevolotsky, Carasso, & Rattner, 1995). As the nutritional quality of grazed diets differs between breeds (Glasser et al., 2012), this provides an opportunity to study the breed-by-diet interactions on milk PL content and composition.

The aim of this study was to investigate the size of the goat MFG and its PL content and composition as influenced by genotype and diet.

2. Material and methods

2.1. Goats, management and diet

Goats were kept according to the Israel Council on Animal Care's 1994 Guidelines. We used 45 goats, including purebred Mamber (n = 12) and Damascus (n = 12), and F_1 crossbreeds Alpine × Mamber (n = 11) and Alpine × Damascus (n = 10). Animals were in their second or third lactation. Allotment to grazing or confinement treatments was based on milk yield in the previous lactation and body weight: each subgroup (confinement or grazing) had an equal proportion of high and low milk producers and of goats of heavy and light weight. Half of each purebred group and the Alpine × Damascus goats were allotted to grazing and half to confinement; for the Alpine × Mamber goats, the allotment was 6 and 5, respectively.

Animals were kept as two groups (grazing and confinement) in a dirt-floored and roofed building. They were milked twice daily at 0600 h and 1400 h, using a OpiFlow™ System for sheep and goats (SCR, Netanya, Israel), whereby a chip is attached to a leg of each animal for identification purposes, allowing for online recording of daily milk yield.

All goats were fed 1.2 kg of a commercial concentrate [16% crude protein, 21.8% NDF and 11.7% acid detergent fibre (ADF); metabolisable energy (ME), 2.80 Mcal kg^{-1}; Ambar, Hadera, Israel] that was provided by an automatic pneumatic dispenser in the milking parlour. The confined group had free access to clover hay [12.9% CP, 61.8%, NDF, 32.3% ADF, 2.23 Mcal ME kg^{-1} dry matter (DM)] when their counterparts were out at pasture. Goats in the grazing group grazed for 4 h daily, not including the time taken to get to the grazing area which is characterised by steep, rocky slopes with sparse patches of shallow soil. The vegetation is dominated by low trees (mainly *Phyllirea latifolia* L.) and tall shrubs (mastic tree, *Pistacia lentiscus* L. and *Calicotome villosa* Poir. Link), *Rubia tenuifolia* D' Urv., *Clematis cirrhosa* L. and *Smilax aspera* L. From January to mid-May, green annual herbaceous vegetation covers the soil patches.

Details on the methodology used to determine feed intake and its composition are provided in the Supplementary Material.

2.2. Milk sampling and analysis

One milk sample was collected once a month at morning milking from each individual goat in the study (n = 45 for each time point). This was done by using the SCR sampling system, which enables sampling throughout an entire milking session. Each sample was divided to three aliquots for each animal; the first (40 mL) was mixed with bronopol (2-bromo-2-nitropropane-1,3-diol and 2-bromo-2-nitropropanol) for analysis of milk fat, protein, lactose and somatic cell count by mid-infrared analysis (standard IDF 141C:2000) at the laboratories of the Israeli Cattle Breeders' Association (Caesarea, Israel). The second aliquot (2 mL) was frozen at −20 °C for analysis of milk TG content and PL composition and content by high performance liquid chromatography. The third aliquot (2 mL) was chilled on ice and used for MFG size analysis no later than 28 h post sampling after validation that MFG size was similar when analysed immediately or 28 h after milking.

2.3. Milk fat globule size

MFG sizes were measured in chilled, but not frozen, milk samples in duplicate using a particle-size analyser with a single modular light-scattering system (Microtrac UPA, North Wales, UK), as previously described (Mesilati-Stahy et al., 2011).

2.4. Lipid extraction and analysis

All reagents used for lipid extraction and analysis, including standards were as detailed before (Argov-Argaman et al., 2014). Retention times were determined by injection of standard commercial mixes of PL and TG.

Total lipids were extracted from the milk using a protocol adapted from the cold-extraction procedure developed by Folch, Lees, and Sloane Stanley (1957), as previously performed in our laboratory (Mesilati-Stahy et al., 2011). Briefly, lipids were extracted and kept in 100 μL chloroform–ethanol (3%, v/v) at −20 °C until further analysis.

Polar and neutral lipids were identified and quantified as previously described (Argov-Argaman et al., 2014). Lipids were identified by the use of external standards: TG, diacylglycerol, monoacylglycerol, free fatty acids, cholesterol, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), PC and SM. Polar and neutral lipids were quantified according to their weight percentages as previously described (Mesilati-Stahy & Argov-Argaman, 2014). Polar lipid and TG calibration curves were calculated by applying the power model equations to the area under the curve and concentration values: