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Altered concentrate to forage ratio in cows ration enhanced bioproduction of specific size subpopulation of milk fat globules

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ABSTRACT

The mechanism underlying the shift in milk-fat-globule (MFG) mean diameter upon changing the concentrate-to-forage ratio in dairy cow rations was investigated. Cows were fed high-concentrate low-forage (HCLF) or high-forage low-concentrate (LCHF) rations for 4 weeks. Mean diameter of MFG, determined in raw whole milk, was 0.4 μm larger in the LCHF-fed vs. HCLF-fed group. The main compositional differences between treatments were found in a specific MFG subgroup with the diameter of 3.3 μm (F1), with higher capric, lauric, myristic and lower oleic acid concentrations in HCLF vs. LCHF milk. Similarly, lipid concentration differences between treatments were only found in F1, with higher triglyceride and phosphatidylethanolamine, and lower sphingomyelin concentrations in LCHF vs. HCLF milk. The higher MFG mean diameter in whole raw LCHF milk might therefore be attributed to increased secretion of F1-group MFG, while fat content and composition in the other MFG size groups remains unchanged.

1. Introduction

Milk fat is secreted from the mammary-gland epithelial cells in a structure termed milk fat globule (MFG), which consists of a triglyceride (TG) core enveloped by a polar lipid trilayer (Mather & Keenan, 1998). The polar lipid envelope of the TG droplet is termed MFG membrane and consists of phospholipids (PL; glycerophospholipids, sphingolipids, glycolipids), cholesterol and proteins, all derived from the endoplasmic reticulum and apical membrane of the mammary epithelium. Therefore, altered composition of the MFG membrane indicates remodeling of the cellular membranes of the secreting mammary epithelial cells.

The MFG are secreted in a variety of sizes ranging from 200 nm to more than 15 μm in humans (Michalski, Briard, Michel, Tasson, & Poulain, 2005) and bovines (Mulder & Walstra, 1974). The weight ratio between TG and PL is determined by the MFG size, with a lower ratio (Mesilati-Stahy & Argov-Argaman, 2014) and higher PL concentration (Lopez et al., 2008) in smaller globules. Aside from differences in biochemical properties, TG and PL differ in their fatty-acid composition, with a higher concentration of saturated fatty acids and lower concentration of polyunsaturated fatty acids (PUFA) in the TG fraction (Bitman & Wood, 1990). In light of these findings, it seems that the mechanism controlling MFG size is also involved in determining the lipid profile (i.e., PL, TG, and fatty acids) of milk.

The MFG-size-dependent lipid composition has important industrial implications. For example, cheese made from large vs. small MFG exhibits different physicochemical characteristics (Michalski et al., 2003). Globule coalescence and aggregation properties of milk are also attributable to MFG-size-dependent lipid composition (Lopez et al., 2011). Moreover, the importance of MFG-size-dependent lipid composition extends beyond physical properties, affecting health and nutrition as well. Milk enriched with small MFG has higher contents of monounsaturated fatty acids (MUFA) and PUFA, which might be of interest due to their beneficial effects on human lipoprotein metabolism. When consumed in the diet, PUFA, and especially omega 3 PUFA, was shown to inhibit atherosclerosis (reviewed by Chang & Deckelbaum, 2013) and modulate immune response (Trevor & Lawrence, 2004). Also, the ratio between omega 6 and omega 3 PUFA in the diet is of great interest since very high omega 6/omega 3 ratio, as is found in Western diets, promote the pathogenesis of various metabolic diseases and even cancer (Simopoulos, 2002). The effect of MUFA in the diet was also studied in relation to metabolic diseases. It was suggested that increased MUFA consumption in the diet reduced low density lipoprotein (LDL) cholesterol content and increased its oxidation resistance capacity (Reviewed by Ulbricht & Southgate, 1991) which may slow the progression of atherosclerosis. The higher PL

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content in small-size MFG (Mesilati-Stahy, Mida, & Argov-Argaman, 2011) might also have health implications; PL are bioactive molecules that are thought to improve plasma lipid profiles and to potentially inhibit colon cancer pathogenesis (Burgess et al., 2005; Dillehay, Webb, Schmelz, & Merril, 1994). Thus, understanding the mechanisms underlying MFG size and composition has multiple nutritional, health, industrial and commercial implications.

The nutritional, evolutionary and biological bases for the secretion of a wide range of MFG sizes are still illusive. However, an association has been found between the composition of some milk constituents and mean diameter of the MFG. For example, MFG size is closely associated with its membrane's PL (Argov et al., 2008; Lopez, Medac, & Jimenez-Flores, 2010; Mesilati-Stahy & Argov-Argaman, 2014; Mesilati-Stahy et al., 2011) and fatty acid composition (Briard, Leconte, Michel, & Michalski, 2003; Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007; Lopez et al., 2008; Mesilati-Stahy, Malka, & Argov-Argaman, 2012; Mesilati-Stahy et al., 2011; Michalski et al., 2006; O'Mahony, Auty, & McSweeney, 2005). Several studies have reported a positive correlation between MFG mean diameter and milk total lipid content (Couvreur et al., 2007; Menard et al., 2010) or with diurnal fat yield (Wiking, Stagsted, Bjorck, & Nielsen, 2004), while others found no positive correlation between fat content and MFG size (King, 1957; Walstra, 1969). Other factors which have been shown to influence MFG structure are genomic predisposition (Argov-Argaman, Mida, Cohen, Visker, & Hettinga, 2013; Couvreur et al., 2007) and diet (Briard et al., 2003; Couvreur et al., 2007; Lopez et al., 2008). Nonetheless, the mechanism underlying the altered mean diameter of the MFG is still illusive. We still do not know what actual structural differences occur under conditions that result in higher MFG mean diameter measured in whole raw milk. It is possible that the increased measured MFG diameter results from secretion of larger MFG on average, leaving their size distribution unchanged. Alternatively, the production of a specific MFG size subpopulation may be accelerated, resulting in a shift in the MFG mean diameter recorded in raw milk. The actual structural differences occur in milk when the recorded MFG mean diameter is altered are important factors defining composition of milk lipids (Mesilati-Stahy et al., 2011). Moreover, the actual MFG size and not the recorded mean diameter may modify industrially important features like ripening and coagulation (Lopez et al., 2011).

The objectives of this project were to study the shift in fat distribution between defined MFG size groups as a possible mechanism for the known diet-induced alteration in MFG mean diameter. The lipid compositions of the distinct MFG size groups were also determined as a factor that might affect MFG structure.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at the Volcani Center experimental farm in Bet Dagan, Israel during the months March to May, which are spring time in Israel. The study was conducted in a crossover design, over 4 weeks. The first week of each period was used as a transition period between treatments. Fresh, never frozen raw milk samples were used to determine the time point at which the greatest differences in MFG mean diameter between treatments was achieved. In the second week of the experiment, the differences recorded between treatments reached 0.4 μm. Therefore, samples obtained during this week were used to determine the fatty acid, PL and TG compositions and concentrations of the various MFG size groups and for statistical analysis.

2.2. Chemicals and reagents

For lipid extraction, methanol and chloroform (both analytical reagent grade) were purchased from Bio-Lab Ltd. (Jerusalem, Israel). For high-performance liquid chromatography (HPLC) analysis, chloroform and ethanol (used at 97:3 v/v, both analytical reagent grade) and methanol (HPLC grade) were purchased from Bio-Lab Ltd. The TG triolein (>99% pure) was purchased from Supelco (Bellefonte, PA). The PL standards were supplied by Sigma Aldrich Ltd. Israel (Rehovot, Israel) and consisted of: PhE (1,2-dioleoyl-sn-glycero-3 phosphoethanolamine, 10 mg PL per ml $CHCl_3$, purity 99%), PI (L-α phosphatidylinositol ammonium salt, from bovine liver, purity 98%), PS (1,2-dioleoyl-sn-glycerol-3-phospho-L-serine sodium salt, purity 95%), PC (1,2-dioleoyl-sn-glycero-3-phosphocholine, purity ⩾99%) and SM (sphingomyelin; from bovine brain, purity 97%); conjugated linoleic acid was used (cis-9 trans-11 linoleic acid, purity >95%) as a standard for free fatty acids. For the gas chromatography (GC) analysis, methanol (analytical reagent grade) was purchased from Bio-Lab, petroleum ether (analytical reagent grade) was from Gadot Lab Supplies (Netanya, Israel) and sulfuric acid was from Bet Dekel (Ra'anana, Israel). Retention times were determined by injection of standard commercial mixes of PL and TG.

2.3. Milk sampling and content analysis

The procedures used in this study were approved by the Volcani Center Animal Care Committee (Ethical clearance number-IL294/10). The experiment was conducted at the Volcani Center experimental farm in Bet Dagan, Israel. Twenty-four Israeli-Holstein cows were randomly allocated to two treatment groups ($n = 12$) with more or less uniform milk yields, days in milk (DIM), parity, age and bodyweight (BW). These 2 groups of cows averaged, respectively, 46.1 ± 4.8 and 45.9 ± 4.8 kg of milk, 155.5 ± 28 and 151.4 ± 27 DIM, parity numbers of 3.1 ± 1.4, 3.1 ± 1.4, and 665.3 ± 35.1, 656.8 ± 50.4 kg BW.

Experimental procedure was aimed to enhance differences in milk fat content between treatments. The treatments were as follows: (1) high-concentrate low-forage diet (HCLF)—cows were fed typical Israeli milking-cow ration consisting of 65% concentrate and 35% forage; (2) low-concentrate high-forage diet (LCHF)—cows were fed a ration consisting of 35% concentrate and 65% forage. The ingredients and chemical composition and the fatty acid composition of both diets are presented in Table 1 and 2, respectively. The study was conducted in a crossover design, with a 4-week experimental period.

Cows were milked 3 times daily and milk yields were recorded electronically at each milking. Cows were also weighed automatically after each milking on a walk-in electronic scale (S.A.E. Afikim, Kibbutz Afikim, Israel). Milk samples were collected weekly from by the Afimilk sampling system, which enables sampling throughout an entire milking session. Samples were aliquoted and the first aliquot was mixed with bronopol (2-bromo-2-nitropropane-1,3-diol and 2-bromo-2-nitropropanol) for component analysis. Milk fat, protein, lactose and SCC were determined by infrared analysis (standard IDF 141C:2000) at the laboratories of the Israeli Cattle Breeders' Association (Caesarea, Israel). A second aliquot was frozen at −20 °C for analysis of milk FA and PL composition by GC and HPLC.

2.4. Gravity-based separation method

The MFG were separated by gravity-based separation method (Ma & Barbano, 2000). Briefly, milk was collected and kept on ice. Samples were transported to the laboratory within 2 h of col-