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Elevated concentrate-to-forage ratio in dairy cow rations is associated with a shift in the diameter of milk fat globules and remodeling of their membranes

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ABSTRACT

We examined the effects of concentrate-to-forage ratio in dairy cow rations on milk-fat composition, with a specific focus on the structure of milk fat globules (MFG). Twenty-four Holstein cows, 153 d in milk, were assigned to 2 dietary treatments in a crossover design study. Treatments were (1) high-concentrate (65%), low-forage (35%; HCLF) diet and (2) low-concentrate (35%), high-forage (65%; LCHF) diet. The mean diameter of the MFG; plasma concentrations of insulin, glucose, and nonesterified fatty acids (FA); and the composition and concentrations of milk FA and polar lipids were determined. Concentrations of insulin were 56% higher, and those of nonesterified FA 46% lower, in the HCLF than in the LCHF diet. The milk yield was 8.5 kg/d higher and yields of fat, protein, and lactose were 180, 350, and 403 g/d higher, respectively, in the HCLF versus LCHF diet. Milk FA composition differed between treatments, with 1.5 and 1.0 percentage units higher saturated and polyunsaturated FA concentrations, respectively, in the HCLF versus LCHF diet. Mean MFG diameter tended to be smaller (0.2 μm) in the HCLF diet than in the LCHF diet, associated with increased daily phospholipids yield (34%), lower phosphatidylserine and higher phosphatidylcholine concentrations. In conclusion, the decreased milk and fat yields in the LCHF diet were associated with remodeling of the MFG membrane and with the secretion of larger MFG. Membrane remodeling of the mammary epithelium membranes seems to play a role in regulating MFG size.

Key words: high-forage diet, milk fat globule, phospholipid, membrane

INTRODUCTION

Milk fat consists of 95 to 98% triglycerides (**TG**) and only 1 to 2% phospholipids (**PL**), depending on animal, diet, and lactation stage (Jensen, 2002). Triglycerides and PL differ in their FA composition, with a higher concentration of SFA and a lower concentration of PUFA in the TG fraction than the PL fraction (Bitman and Wood, 1990). Due to their different FA compositions and molecular structures, when ingested as part of the human diet, TG and PL induce different metabolic and physiological responses, with health-promoting responses attributed to the PL. For example, dietary supplementation of 2.5 to 5% PL is associated with a variety of health-promoting bioactivities, such as improved plasma lipid profile, decreased postprandial cholesterol absorption, and even reduced rates of colon cancer pathogenesis (Dillehay et al., 1994; Burgess et al., 2005). Therefore, health and nutritional implications exist for understanding how the relative concentrations of PL in milk are determined.

Milk fat is secreted in a unique structure termed milk fat globule (**MFG**). The MFG consists of a TG core covered with 3 layers of PL, termed the MFG membrane (**MFGM**; Mather and Keenan, 1998). The size distribution of MFG in bovine milk ranges over 3 orders of magnitude, from less than 200 nm to over 15 μm (Mulder and Walstra, 1974). The weight ratio between TG and PL is determined by the MFG size, with a lower ratio (Mesilati-Stahy and Argov-Argaman, 2014) and higher PL concentration (Lopez et al., 2008) in smaller globules. Therefore, the mechanisms controlling MFG size also determine the lipid profile of milk in terms of both bioactive lipid components such as PL and unsaturated FA, and hence can alter the milk dietary and health properties. The size of the MFG also influences the moisture content of dairy products (Michalski et al., 2004), dairy product rennet gel properties (Michalski et al., 2002), and cheese firmness (Michalski et al., 2003). Therefore, the importance of MFG size extends beyond its health implications to dairy-product quality.

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The mechanisms governing the size variations of MFG are not clear. Milk total fat content has been suggested as a predictor of MFG mean diameter (El-Zeini, 2006), because the 2 factors are positively correlated, as also suggested by others (Couvreur et al., 2007; Lopez et al., 2008; Ménard et al., 2010). Also, dietary treatments that are expected to change milk fat content, such as n-3 FA supplementation and pasture compared with a corn silage-based diet (Avramis et al., 2003; Wiking et al., 2003; Couvreur et al., 2007), were also associated with altered MFG size. However, other studies have shown no significant correlation between fat content and MFG size (King, 1957; Walstra, 1969; Wiking et al., 2004).

Other parameters that have been shown to modulate milk fat content and MFG size are lactation stage (Mesilati-Stahy and Argov-Argaman, 2014) and genetic disposition (Couvreur et al., 2007; Argov-Argaman et al., 2013). Interestingly, conditions that induce changes in MFG mean diameter also change milk PL content and composition (Lopez et al., 2008; Argov-Argaman et al., 2012; Mesilati-Stahy et al., 2012), suggesting a link between the metabolic pathways regulating the composition of the mammary gland cell membranes and MFG size. The effect of altered composition of MFGM on MFG size is still unclear, although in other cell models, regulation of intracellular lipid droplet size through altered membrane composition has been demonstrated (reviewed by Thiam et al., 2013).

Our hypothesis was that modulation of milk fat yield and concentration in response to ration composition would be associated with altered MFG mean diameter, possibly due to changes in MFG PL composition. This would allow us to study the structural regulation of MFG. Modulation of milk fat yield and concentration was achieved by changing the forage-to-concentrate ratio in dairy cow rations.

MATERIALS AND METHODS

Milk Sampling and Content Analysis

The procedures used in this study were approved by the Volcani Center Animal Care Committee. The experiment was conducted at the Volcani Center experimental farm in Bet Dagan, Israel. Twenty-four Israeli-Holstein cows were housed in covered loose pens with adjacent outside yards and were divided into 2 treatment groups by milk yields, DIM, parity, and BW. These 2 groups of cows averaged (mean ± SD), respectively, 46.1 ± 4.8 and 45.9 ± 4.8 kg of milk/d, 155.5 ± 28 and 151.4 ± 27 DIM, parities 3.1 ± 1.4 and 3.1 ± 1.4, and 665.3 ± 35.1 and 656.8 ± 50.4 kg of BW.

The treatments were as follows: (1) high-concentrate, low-forage (**HCLF**) diet—cows were fed typical Israeli milking-cow rations consisting of 65% concentrate and 35% forage; and (2) low-concentrate, high-forage (**LCHF**) diet—cows were fed a ration consisting of 35% concentrate and 65% forage. The composition of both diets is presented in Table 1. The study was conducted in a crossover design, with a 4-wk 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 walking electronic scale (S.A.E. Afikim, Kibbutz Afikim, Israel). Milk samples were collected weekly from 3 consecutive milking sessions by the Afimilk sampling system (S.A.E. Afikim), which enables sampling throughout an entire milking session. A composite from each day for each cow was collected proportional to the milk yield at each individual milking. This protocol allowed us to overcome the variations in milk solids composition that can occur throughout the day. The representative sample was 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, and lactose contents were determined by infrared analysis (IDF, 2000) at the laboratories of the Israeli Cattle Breeders' Association (Caesarea, Israel). Also, SCC was determined in fresh milk samples. A second aliquot was frozen at −20°C for analysis of milk FA and PL composition by gas chromatography and HPLC.

Blood samples were taken twice per week (Monday and Wednesday) 2 h before feeding throughout the study from the coccygeal vein, using vacuum tubes containing lithium heparin (BD, Plymouth, UK). The tubes were immediately placed on ice and centrifuged at 3,000 × *g* for 15 min at room temperature. Plasma was separated and frozen at −32°C pending analysis.

Chemical Analyses of Plasma Hormones and Metabolites

Plasma insulin concentration was determined by RIA (Diagnostic Products Corp., Los Angeles, CA), with intra- and interassay coefficients of variation of 7.2 and 5.1%, respectively. Plasma glucose concentration was determined with a glucose reagent kit (Glucose UV 10 × 50 mL; Raichem, San Diego, CA), with intra- and interassay coefficients of variation of 2.8 and 2.3%, respectively. Plasma NEFA concentrations were determined with the Wako NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany), with intra- and interassay coefficients of variation of 7.1 and 6.3%, respectively.