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Dietary fish oil supplements depress milk fat yield and alter milk fatty acid composition in lactating cows fed grass silage-based diets

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ABSTRACT

The potential of dietary fish oil (FO) supplements to increase milk 20:5n-3 and 22:6n-3 concentrations and the associated effects on milk fatty acid (FA) composition, intake, and milk production were examined. Four multiparous lactating cows offered a grass silage-based diet (forage:concentrate ratio 58:42, on a dry matter basis) supplemented with 0, 75, 150, or 300 g of FO/d (FO0, FO75, FO150, and FO300, respectively) were used in a 4 × 4 Latin square with 28-d experimental periods. Milk FA composition was analyzed by complementary silver-ion thin-layer chromatography, gas chromatography-mass spectrometry, and silver-ion HPLC. Supplements of FO decreased linearly dry matter intake, yields of energy-corrected milk, milk fat and protein, and milk fat content. Compared with FO0, milk fat content and yield were decreased by 30.1 and 40.6%, respectively, on the FO300 treatment. Supplements of FO linearly increased milk 20:5n-3 and 22:6n-3 concentrations from 0.07 to 0.18 and 0.03 to 0.10 g/100 g of FA, respectively. Enrichment of 20:5n-3 and 22:6n-3 was accompanied by decreases in 4- to 18-carbon saturated FA and increases in total conjugated linoleic acid (CLA), *trans* FA, and polyunsaturated FA concentrations. Fish oil elevated milk fat *cis*-9,*trans*-11 CLA content in a quadratic manner, reaching a maximum on FO150 (from 0.61 to 2.15 g/100 g of FA), whereas further amounts of FO increased *trans*-10 18:1 with no change in *trans*-11 18:1 concentration. Supplements of FO also resulted in a dose-dependent appearance of 37 unique 20- and 22-carbon intermediates in milk fat. Concentrations of 16-, 18-, 20-, and 22-carbon *trans* FA were all increased by FO, with enrichment of *trans* 18:1 and *trans* 18:2 being quantitatively the most important. Decreases in milk fat yield to FO were not related to changes in milk *trans*-10,*cis*-12 CLA concentration or estimated milk fat melting point. Partial least square regression analysis indicated that FO-induced milk fat depression was associated with changes in the concentrations of multiple FA in milk. Even though a direct cause and effect could not be established, a decrease in 18:0 supply in combination with increased mammary uptake of *cis*-11 18:1, *trans*-10 18:1, and *trans* 20- and 22-carbon FA may contribute. In conclusion, dietary FO supplements enrich 20:5n-3 and 22:6n-3 in milk, but also elevate mono- and polyenoic *trans* FA concentrations, and in high amounts alter the distribution of individual *trans* FA isomers.

Key words: conjugated linoleic acid, fish oil, milk fat, *trans* fatty acid

INTRODUCTION

Increasing evidence has shown that nutrition is an important factor involved in the onset and development of several chronic diseases in humans, including cancer, cardiovascular disease (**CVD**), type II diabetes, and obesity. Clinical studies implicate an excessive consumption of medium-chain SFA and *trans* FA as risk factors for CVD and in the etiology of insulin resistance (WHO, 2003; Shingfield et al., 2008). Observational, prospective cohort, and randomized intervention studies have contributed to a body of evidence to indicate that moderate increases in the consumption of the long-chain n-3 PUFA, 20:5n-3 and 22:6n-3, lowers CVD disease risk, decreases the incidence of sudden cardiac arrest, improves immune function, and may prevent certain cancers (Palmquist, 2009).

Milk and dairy products are a major source of 12:0, 14:0, 16:0, and total SFA in the Western diet, but also contain several other FA, including 4:0, several odd- and branched-chain FA, *cis*-9 18:1, and *cis*-9,*trans*-11 CLA,

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with potential to prevent the onset and development of chronic disease (Lock and Bauman, 2004; Shingfield et al., 2008). Consequently, considerable interest exists in producing milk and dairy foods containing lower amounts of medium-chain SFA and higher concentrations of *cis*-9 18:1, *cis*-9,*trans*-11 CLA, and long-chain n-3 PUFA for improving human health without requiring changes in consumer eating habits.

Dietary supplements of fish oil (**FO**) can be used to increase long-chain n-3 PUFA concentrations in ruminant milk, but enrichment varies depending on the basal diet and source of marine lipid (Chilliard et al., 2001; Shingfield et al., 2013). Supplements of FO are also effective for enriching milk fat *cis*-9,*trans*-11 CLA concentrations, increases that are also accompanied by elevated proportions of *trans* 18:1 and *trans* 18:2 isomers (Chilliard et al., 2001; Shingfield et al., 2003; Loor et al., 2005). Recent reports indicate that the FA in FO also modify ruminal biohydrogenation resulting in the formation and accumulation of 20- and 22-carbon FA containing at least a single *trans* double bond in sheep (Toral et al., 2010) and cattle (Kairenius et al., 2011; Shingfield et al., 2012), but the occurrence of these intermediates in bovine milk fat has not been documented.

Supplements of FO typically decrease milk fat synthesis in lactating cows (Offer et al., 1999; Donovan et al., 2000; Keady et al., 2000), a response associated with the effects of FA in FO on ruminal lipid metabolism (Loor et al., 2005). The exact cause of FO-induced milk fat depression (**MFD**) in lactating cows has not been established, but several hypotheses have been postulated, including increased formation of biohydrogenation intermediates that inhibit milk fat synthesis, lowered availability of 18:0 for endogenous *cis*-9 18:1 synthesis, and an increase in milk fat melting point (Loor et al., 2005; Shingfield and Griinari, 2007; Gama et al., 2008).

The objectives of the present investigation were (1) to evaluate the potential of dietary FO supplements to enrich milk 20:5n-3 and 22:6n-3 concentrations and the associated effects on milk FA composition and animal performance, and (2) examine the relationships between the flow of FA at the omasum, changes in milk FA composition, and milk fat synthesis to provide further insight into the possible causes of FO-induced MFD. Chemical composition of dietary ingredients and treatment effects on FA intake, ruminal microbial ecology, rumen fermentation characteristics, and flow of FA at the omasum have been reported previously (Shingfield et al., 2012).

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

All experimental procedures were approved by the Animal Ethics Committee of MTT Agrifood Research Finland (Jokioinen, Finland) in accordance with the Use of Vertebrates for Scientific Purposes Act of 1985. Four multiparous (2 Finnish Ayrshire and 2 Friesian) cows fitted with a rumen cannula (i.d. 100 mm; Bar Diamond Inc., Parma, ID), averaging 578 ± 13.6 kg of BW, 159 ± 28.5 DIM, and producing 22.3 ± 2.42 kg of milk/d were allocated at random to experimental treatments according to a 4 × 4 Latin square with 28-d periods. A detailed description of animal management and experimental treatments is reported elsewhere (Shingfield et al., 2006, 2012). In brief, cows were offered grass silage and a cereal-based concentrate (forage:concentrate ratio 58:42, on a DM basis) as equal meals at 0600 and 1800 h in an amount representing 95% of ad libitum intake measured over a period of 14 d immediately before the start of the experiment. Treatments comprised 0, 75, 150, and 300 g/d of ultrarefined herring and mackerel oil (EPAX 3000 TG, Pronova Biocare AS, Aalesund, Norway; designated as treatments **FO0**, **FO75**, **FO150**, and **FO300**, respectively), offered in equal amounts by mixing thoroughly with concentrate ingredients just before feeding. Cows were housed in individual tiestalls within a dedicated metabolism unit with continuous access to water and milked twice daily at 0700 and 1645 h.

Measurements, Sampling, and Chemical Analysis

Intake and milk yield were measured daily throughout the experiment. Samples of milk were collected over 2 consecutive milkings starting at 1645 h on d 17, 20, 24, and 27 of each experimental period, treated with preservative (Bronopol, Valio Ltd., Helsinki, Finland), and analyzed for milk fat, CP, and lactose by mid-infrared spectroscopy (Milko-Scan 133B, Foss Electric, Hillerød, Denmark). Unpreserved milk samples were also collected at the same time, stored at −20°C, and composited according to milk yield until analyzed for FA composition. Ruminal administration of LiCo-EDTA, Yb-acetate, and Cr-mordanted straw as indigestible markers to estimate flow of FA at the omasum were found to alter milk fat composition (Shingfield et al., 2006); therefore, only samples of milk collected immediately before marker administration were submitted for detailed FA analysis.