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Short communication: Effects of diets containing supplemental fats on ruminal fermentation and milk odd- and branched-chain fatty acids in dairy cows

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ABSTRACT

There is a growing interest in odd- and branched-chain fatty acids (OBCFA) in milk following reports that several branched-chain fatty acids (FA) have health promoting effects, and certain milk OBCFA could serve as a biomarker to assess ruminal function. Twenty-four Holstein cows were fed 3 low-forage diets containing 30 g/kg of dry matter of prilled palm fat (PPF), sunflower oil (SO), or an equal mixture of both fats (experiment 1) or 3 diets containing 30 g/kg of dry matter of SO with a forage-to-concentrate ratio of 39:61, 44:56, or 48:52 (Experiment 2); diets were fed to investigate milk OBCFA composition and to explore the relationships between ruminal VFA and milk OBCFA using principal component analysis. Including SO in diets decreased yields of milk 13:0 *anteiso*, 15:0 *anteiso*, 15:0, 17:0, *cis*-9 15:1, and *cis*-9 17:1 compared with PPF. The molar proportion of ruminal propionate was the lowest and the yields of milk 14:0 *iso* and 16:0 *iso* were the greatest with the diet containing both fat supplements. Replacing concentrate with forages linearly increased ruminal acetate and yields of milk 13:0 *iso*, 14:0 *iso*, 15:0 *iso*, 16:0 *iso*, 17:0 *iso*, 13:0 *anteiso*, 15:0 *anteiso*, 15:0, 17:0, *cis*-9 15:1, and *cis*-9 17:1. The principal component analysis revealed that ruminal molar proportion of acetate related to concentrations of milk *iso* FA containing <17-carbon, whereas ruminal propionate related to milk 15:0, 17:0, *cis*-9 15:1, and *cis*-9 17:1, with the stronger correlations between milk OBCFA and ruminal acetate than propionate. No associations were found between ruminal molar proportion of butyrate and milk OBCFA concentrations. The results suggest that complete replacement of PPF with SO at 30 g/kg of dry matter in low-forage diets is not an effective strategy to enhance bioactive branched-chain FA in milk, rather this feeding practice lowers *anteiso* FA in milk; however, increasing forage proportion in diets containing SO enhances several *iso* and *anteiso* FA in milk. The milk OBCFA concentrations have stronger correlations with ruminal acetate molar proportion than with propionate or butyrate in cows fed diets containing supplemental fats.
Key words: forage proportion, *iso* and *anteiso* fatty acid, palm fat, sunflower oil, volatile fatty acid

Short Communication

Fat supplements are often added to the diets of lactating cows to increase energy concentration and milk production. Fats rich in SFA increase SFA concentrations in milk (Lock et al., 2013). Inclusion of unsaturated fat sources in the diets lowers SFA and enhances UFA in milk, and those rich in 18:2n-6 and 18:3n-3 are the most effective to increase *cis*-9,*trans*-11 CLA (Shingfield et al., 2013), which improves concentrations of health promoting fatty acids (**FA**) in milk. Interest has been growing in enriching ruminant-derived foods with branched-chain FA, because several *iso* and *anteiso* FA have shown many beneficial effects, including inhibition of the growth of several cancer cell lines in vitro and maintenance of enterocyte health (Ran-Ressler et al., 2014).

Milk fat branched-chain FA originate principally from the digestion and absorption of ruminal microbial lipids (Fievez et al., 2012). Changes in milk branched-chain FA in response to fat supplements are not well characterized; however, fat supplementation may change these milk FA (Baumann et al., 2016) via modifying the ruminal microbial populations (Bayat et

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al., 2018). Moreover, the magnitude of responses likely depends on amount, form, and FA profile of dietary fats and the composition of basal diets. Dietary inclusion of plant oils rich in 18:2n-6 reduced concentrations of several *iso* and *anteiso* FA in milk in grass silage diets (Halmemies-Beauchet-Filleau et al., 2011), but not in mixture of corn silage and grass silage diets (Alfonso-Avila et al., 2017). One potential strategy to enhance branched-chain FA, in particular *iso* FA, in milk is increasing dietary forage proportion (Fievez et al., 2012; Patel et al., 2013); however, examining forage level in diets containing fat supplements on these FA in milk is limited (Saliba et al., 2014).

Milk odd- and branched-chain FA (**OBCFA**) have been used to predict the molar proportions of individual ruminal VFA as a noninvasive tool to assess ruminal fermentation function (Bhagwat et al., 2012); however, some reports do not completely support application of milk OBCFA to estimate ruminal VFA due to postruminal modification of OBCFA composition (French et al., 2012; Vlaeminck et al., 2015). It has been indicated that *iso* FA and straight odd-chain FA in milk have positive correlations with ruminal acetate and propionate, respectively (Fievez et al., 2012); however, the associations between ruminal fermentation pattern and milk OBCFA composition have not been well examined for diets containing fat supplements.

The purpose of our study was to evaluate the effects of substituting a saturated with an unsaturated fat supplement in low-forage diets on branched-chain FA in milk; however, it is well established that inclusion of unsaturated fat in high-concentrate diets induces milk fat depression (Harvatine et al., 2009). Therefore, the other aim of our study was to examine increasing forage proportions in diets containing a supplemental unsaturated fat as a potential strategy to simultaneously recover milk fat depression and to enhance health beneficial FA in milk including branched-chain FA secretions. It was hypothesized that milk OBCFA could be altered due to modifications of ruminal microbial populations by dietary supplemental fats and forage proportions. We examined the effects of replacement of a saturated fat-enriched 16:0 (prilled palm fat; **PPF**) with an unsaturated fat rich in 18:2n-6 (sunflower oil; **SO**) in low-forage diets (experiment 1) or increasing the forage-to-concentrate ratio (**F:C**) in diets containing SO (experiment 2) on ruminal fermentation characteristics and milk OBCFA composition. The dietary effects on feed intake, digestibility, milk production and composition, and milk FA composition, except OBCFA, were reported previously (Vazirigohar et al., 2014). Moreover, data from both experiments were used to explore the associations among ruminal VFA and milk OBCFA in cows fed diets containing supplemental fats using principal component analysis (**PCA**).

All experimental procedures were approved by the Animal Experiment Committee of the University of Tehran (Alborz, Iran). Twenty-four multiparous Holstein cows from the Dairy Research Farm of the University of Tehran were used in experiment 1 (mean ± SD; 618 ± 9.19 kg of BW, 3.2 ± 0.22 parity, 90 ± 6.04 DIM, and 35.8 ± 0.88 kg of milk/d). Experiment 2 started after a 14-d washout period using 19 out of 24 cows recruited from experiment 1 and 5 additional cows (619 ± 15.1 kg of BW, 3.0 ± 0.24 parity, 107 ± 6.22 DIM, and 35.5 ± 0.89 kg of milk/d). During the washout period, all cows received a TMR containing alfalfa hay and corn silage (5:4 wt/wt; on DM basis), concentrate (F:C of 39:61; on DM basis), and 18 g/kg of DM of PPF. Cows in each experiment were blocked by DIM and then allocated to treatments according to a randomized complete block design with 3-d baseline, 18-d adaptation, and 7-d measurement periods (total 25-d experimental period). Treatments in experiment 1 consisted of low-forage (F:C of 39:61, DM basis) diets containing 30 g/kg of DM of either (1) PPF (fractionated refined palm oil; RumiFat R100 from Ecolex, Selangor, Malaysia), (2) SO (refined SO; Oila-Golrang Pakhsh Co., Tehran, Iran), or (3) an equal mixture of both fats (Table 1). Treatments in experiment 2 consisted of either (1) low-forage (F:C of 39:61), (2) medium-forage (F:C of 44:56), or (3) high-forage (F:C of 48:52) diets containing 30 g/kg of DM of SO (Table 1). Cows were housed in individual tiestalls with ad libitum access to water and milked thrice daily (0100, 1000, and 1700 h). The TMR was offered daily as equal meals at 0800 and 1600 h ensuring ad libitum intakes (i.e., 10% orts as fed).

Samples of milk (without preservative) were collected at each milking on d 19, 21, and 23 of each experiment, and stored at −20°C until analysis of FA. Samples of ruminal fluid were collected on d 25 within 3 to 5 h after morning feeding using a stomach tube attached to an Erlenmeyer flask connected to a vacuum pump. An initial 200 mL of ruminal fluid sample was discarded to minimize salivary contamination. After collection (300-mL sample), samples were filtered through 4 layers of cheesecloth and pH was determined immediately using a portable pH-meter (827 pH Lab; Metrohm AG, Herisau, Switzerland). A 10-mL aliquot of the filtered ruminal fluid was acidified with 1 mL of 50% metaphosphoric acid and stored at −20°C for VFA analysis. Ruminal fluid samples were collected on the last day of each experiment, after completing the collection of milk samples, to avoid potential effects of stress on cows and consequently on the validity of milk FA composition.