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## Plant oil supplements reduce methane emissions and improve milk fatty acid composition in dairy cows fed grass silage-based diets without affecting milk yield

A. R. Bayat,<sup>\*1</sup> I. Tapio,<sup>†</sup> J. Vilkkki,<sup>†</sup> K. J. Shingfield,<sup>\*2</sup> and H. Leskinen<sup>\*</sup>

<sup>\*</sup>Milk Production Solutions, and

<sup>†</sup>Animal Genomics, Green Technology, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland

### ABSTRACT

Four lipid supplements varying in chain length or degree of unsaturation were examined for their effects on milk yield and composition, ruminal CH<sub>4</sub> emissions, rumen fermentation, nutrient utilization, and microbial ecology in lactating dairy cows. Five Nordic Red cows fitted with rumen cannulas were used in a 5 × 5 Latin square with five 28-d periods. Treatments comprised total mixed rations based on grass silage with a forage-to-concentrate ratio of 60:40 supplemented with no lipid (CO) or 50 g/kg of diet dry matter (DM) of myristic acid (MA), rapeseed oil (RO), safflower oil (SO), or linseed oil (LO). Feeding MA resulted in the lowest DM intake, and feeding RO reduced DM intake compared with CO. Feeding MA reduced the yields of milk, milk constituents, and energy-corrected milk. Plant oils did not influence yields of milk and milk constituents, but reduced milk protein content compared with CO. Treatments had no effect on rumen fermentation characteristics, other than an increase in ammonia-N concentration due to feeding MA, RO, and SO compared with CO. Lipid supplements reduced daily ruminal CH<sub>4</sub> emission; however, the response was to some extent a result of lower feed intake. Lipids modified microbial community structure without affecting total counts of bacteria, archaea, and ciliate protozoa. Dietary treatments had no effect on the apparent total tract digestibility of organic matter, fiber, and gross energy. Treatments did not affect either energy secreted in milk as a proportion of energy intake or efficiency of dietary N utilization. All lipids lowered de novo fatty acid synthesis in the mammary gland. Plant oils increased proportions of milk fat 18:0, *cis* 18:1, *trans* and mono-

unsaturated fatty acids, and decreased saturated fatty acids compared with CO and MA. Both SO and LO increased the proportion of total polyunsaturated fatty acids, total conjugated linolenic acid, and *cis*-9,*trans*-11 conjugated linoleic acid. Feeding MA clearly increased the  $\Delta^9$  desaturation of fatty acids. Our results provide compelling evidence that plant oils supplemented to a grass silage-based diet reduce ruminal CH<sub>4</sub> emission and milk saturated fatty acids, and increase the proportion of unsaturated fatty acids and total conjugated linoleic acid while not interfering with digestibility, rumen fermentation, rumen microbial quantities, or milk production.

**Key words:** lipids, methane, milk fatty acids, microbial diversity

### INTRODUCTION

It is anticipated that global demand for animal-derived food will increase in the coming decades, mainly due to population growth and income rise in developing countries (Bodirsky et al., 2015). Ruminants contribute to high-quality food production by utilizing inedible plant resources, such as forages and agricultural by-products. The livestock sector also contributes to rural development, the rural economy, and maintenance of ecosystems, among many other benefits. However, the livestock sector also plays an important role in climate change, contributing 14.5% of anthropogenic greenhouse gas (GHG) emissions (estimated 7.1 Gt of CO<sub>2</sub> equivalents per year; Gerber et al., 2013). Several nutritional and management strategies have been reported to be effective in mitigation of GHG emissions from animal production systems (Knapp et al., 2014). Practical supplementation levels of plant oil and oilseeds (<80 g/kg of total lipid in diet) can lower GHG consistently over time without compromising the performance of growing or lactating cattle; however, at higher levels, feed intake is compromised (Grainger and Beauchemin, 2011).

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<sup>1</sup>Corresponding author: [alireza.bayat@luke.fi](mailto:alireza.bayat@luke.fi)

<sup>2</sup>Deceased September 11, 2016.

Lipid supplements have been used to increase diet energy density of lactating dairy cows to meet the high energy requirements during early and mid lactation. Dietary lipid supplements are also arguably one of the effective means to lower ruminal CH<sub>4</sub> emissions in ruminants (Martin et al., 2010). Dietary supplements of medium-chain SFA (e.g., lauric and myristic acids) have shown potential to reduce CH<sub>4</sub> emissions in dairy cows (Odongo et al., 2007; Hollmann et al., 2012). Dietary supplements of plant oils rich in MUFA or PUFA have been shown to reduce CH<sub>4</sub> emissions in growing cattle (Beauchemin and McGinn, 2006) and lactating cows (Martin et al., 2010); however, it is crucial to avoid reducing feed intake due to dietary lipid supplementation. One or more mechanisms are thought to contribute to the decrease in CH<sub>4</sub> production to dietary lipid supplements, including lowered digestion of OM in the rumen, shifting rumen fermentation from acetate toward propionate production, and direct inhibitory effects on methanogens and protozoa (Martin et al., 2010). Lower feed intake and replacement of fermentable OM by lipids also contributes to lower CH<sub>4</sub> production (Knapp et al., 2014). However, changes in the rumen microbial community associated with decreased ruminal CH<sub>4</sub> production to lipid supplements are not well characterized. Recent advances in sequencing methodology have made it possible to use 16S to 18S rRNA gene sequences to describe microbial communities under normal rumen conditions (Henderson et al., 2015).

Milk and dairy products are a major source of SFA in the human diet, but serve also as a versatile source of nutrients (Kliem and Shingfield, 2016). Altering the fatty acid composition of milk offers an opportunity to lower consumption of SFA without losing the benefits of other nutrients and bioactive lipids present in milk. Interest exists in manipulating milk fatty acid composition by reducing SFA and increasing UFA or CLA through dairy cow nutrition, especially dietary lipid supplementation (Dewhurst et al., 2006), as well as decreasing the ratio of omega-6 to omega-3 fatty acids (Welter et al., 2016). Both type and source of lipid supplement together with the composition of the basal diet can affect the composition of these fatty acids in milk (Dewhurst et al., 2006). Although inclusion of medium-chain SFA in the diet elevates their contents in milk (Jordan et al., 2006; Machmüller, 2006; Hristov et al., 2009), they are considered unhealthy for humans; thus, dietary plant oil and oilseed supplements can improve milk fatty acid composition to meet the consumers' demand (Dewhurst et al., 2006; Welter 2016).

The aim of the current experiment was to evaluate suitability of dietary lipid supplementation as a CH<sub>4</sub>-mitigation strategy that can simultaneously improve

milk fatty acid composition using diets based on restrictively fermented grass silage. The study examined the effects of dietary supplements of a medium-chain fatty acid (myristic acid) or long-chain UFA sources rich in *cis*-9 18:1, 18:2n-6, and 18:3n-3 (rapeseed, safflower, and linseed oil, respectively) on intake, milk production and composition, milk fatty acid composition, nutrient digestion, ruminal CH<sub>4</sub> emissions, and microbial ecology.

## MATERIALS AND METHODS

### *Animals, Experimental Design, and Diets*

National Ethics Committee (ESAVI/576/04.10.03/2012, Hämeenlinna, Finland) approved all experimental procedures in accordance with the guidelines established by the European Community Council Directives 86/609/EEC (European Union, 1986). Five multiparous Nordic Red dairy cows in early lactation fitted with rumen cannula (#1C, i.d. 100 mm; Bar Diamond Inc., Parma, ID) of 63 ± 5.2 DIM (mean ± SE), 705 ± 11.4 kg BW, 4.0 ± 0.3 parity, and producing 35.2 ± 1.3 kg of milk/d were used in a 5 × 5 Latin square with 28-d experimental periods comprising 15 d of adaptation, 8 d of sampling, and 5 d of washout in each experimental period to minimize treatment carryover effects.

Cows were housed in individual tiestalls, had free access to water and salt block, and were milked twice daily at 0700 and 1645 h. Treatments comprised a basal diet (CO), or the basal diet supplemented with 50 g/kg of diet DM of methyl esters of myristic acid (MA; Sigma-Aldrich, St. Louis, MO), rapeseed oil (RO; Raisioagro Ltd., Raisio, Finland), safflower oil (SO; Statfold Seed Oil Ltd., Staffordshire, UK), or linseed oil (LO; Elix Oil Ltd., Somero, Finland). Lipid supplements replaced barley grain and molassed sugar beet pulp and were introduced to the diet gradually over the first 3 d of each period. The level of lipid supplementation (50 g/kg of DM) was chosen to benefit from maximum effects on milk fatty acids and enteric CH<sub>4</sub> without compromising the DMI of the cows. Diets were fed as 5 equal meals at 0600, 0900, 1200, 1630, and 1930 h. The basal diet was a TMR with a 60:40 forage-to-concentrate ratio on a DM basis (Table 1). Diets were formulated to be isonitrogenous and fed as TMR to avoid selection of dietary components and to maintain the desired forage-to-concentrate ratio. The forage was a mixture of 2 grass silages grown at Jokioinen (60°49'N, 23°28'E), prepared from first and second cut (50:50 on a DM basis, respectively) of timothy and meadow fescue (54:46), and ensiled with a formic acid-based ensiling additive (AIV2 plus, 5 L/t; AIV Valio Ltd., Helsinki, Finland).

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