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Effect of dietary fish oil supplements alone or in combination with sunflower and linseed oil on ruminal lipid metabolism and bacterial populations in lactating cows

P. Kairenius,*[1] H. Leskinen,* V. Toivonen,* S. Muetzel,† S. Ahvenjärvi,* A. Vanhatalo,‡ P. Huhtanen,*§ R. J. Wallace,# and K. J. Shingfield*[2]
*Milk Production Solutions, Green Technology, Natural Resources Institute Finland (Luke), Jokioinen, FI-31600, Finland
†AgResearch Ltd., Grasslands Research Centre, Private Bag 11-008, Palmerston North 4442, New Zealand
‡Department of Agricultural Sciences, University of Helsinki, PO Box 28, FI 00014, Finland
§Department of Agriculture in Northern Sweden, Swedish University of Agricultural Sciences, S-90183, Umeå, Sweden
#Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, Aberdeen, AB21 9SB, United Kingdom

ABSTRACT

Fish oil (FO) alters ruminal biohydrogenation causing *trans* fatty acid (FA) intermediates to accumulate, but the effects of 18-carbon polyunsaturated FA supply on ruminal long-chain FA metabolism and microbial communities in cattle fed FO are not well established. Four cows fitted with rumen cannula were used in a 4 × 4 Latin square with 21-d experimental periods to evaluate the effects of FO alone or in combination with plant oils high in 18:2n-6 or 18:3n-3 on rumen microbial ecology and flow of FA at the omasum. Treatments comprised a basal grass silage-based diet containing no additional oil (control) or supplements of FO (200 g/d) or FO (200 g/d) plus 500 g/d of sunflower oil (SFO) or linseed oil (LFO). Flow of FA was determined using the omasal sampling technique. The relative abundance of key biohydrogenating bacteria was assessed by quantitative PCR on 16S rRNA genes in omasal digesta. Fish oil-supplemented treatments increased the amounts of *trans*-18:1, *trans*-18:2, and 20- to 22-carbon polyunsaturated FA escaping the rumen. Relative to the control, oil supplements had no effect on the amount of 18:0 leaving the rumen, but LFO decreased the flow of 18:0 at the omasum compared with SFO. Both SFO and LFO increased *trans*-18:1 relative to FO, whereas LFO resulted in the highest *trans*-18:2 and 20- to 22-carbon FA flow. Supplements of FO plus plant oils shifted biohydrogenation toward *trans*-10 18:1 formation. Compared with FO alone, the ruminal metabolism of 22:6n-3 in the rumen of lactating cows is more extensive on diets containing higher amounts of 18-carbon polyunsaturated FA. However, the biohydrogenation of 22:5n-3 was less extensive in LFO than SFO, but showed no difference between FO and diets containing plant oils. Ruminal outflow of 20:5n-3 was not altered when plant oils were added to FO. Alterations in the amount of intermediates at the omasum or ruminal biohydrogenation pathways were not accompanied by major changes in analyzed bacterial populations. In conclusion, dietary supplements of FO alone or in combination with plant oils increase the amount of biohydrogenation intermediates containing 1 or more *trans* double bonds escaping the rumen, which may have implications for host metabolism and the nutritional quality of ruminant foods.
Key words: polyunsaturated fatty acid, plant oil, biohydrogenation, *Butyrivibrio*

INTRODUCTION

Due to the potential benefits for human health, there is interest in increasing *cis*-9,*trans*-11 CLA and long-chain n-3 fatty acids (**FA**) and lowering the concentration of medium-chain FA in ruminant milk (Lock and Bauman, 2004; Palmquist et al., 2005; Shingfield et al., 2013). Most of the *cis*-9,*trans*-11 CLA in milk originates from the desaturation of *trans*-11 18:1 in the mammary gland (Griinari et al., 2000). *Trans*-11 18:1 is the penultimate intermediate formed during the biohydrogenation of 18-carbon PUFA to 18:0 in the rumen (Harfoot and Hazlewood, 1988). Dietary supplements of plant oils high in 18:2n-6 (Shingfield et al., 2008a) and 18:3n-3 (Loor et al., 2004) or fish oil (**FO**; Shingfield et al., 2003) are known to increase *trans*-11 18:1 formation in the rumen in lactating cows. Feeding diets containing FO and sources of 18:2n-6 or 18:3n-3 increase *cis*-9,*trans*-11 CLA concentrations in milk from lactating cows (Whitlock et al., 2002; Palmquist and Griinari, 2006), but enrichment varies depending

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[1]Corresponding author: piia.kairenius@luke.fi
[2]Deceased.

on the composition of the basal diet and the amount and source of lipid supplement. Furthermore, dietary supplements of oils containing 18:2n-6 or 18:3n-3, but not FO, lower the proportions of 12:0, 14:0, and 16:0 in milk fat (Shingfield et al., 2013).

In vitro studies have demonstrated that 18:2n-6 and 18:3n-3 decrease the biohydrogenation of 20:5n-3 and 22:6n-3 and cause *trans*-11 18:1 to accumulate (Chow et al., 2004; Wasowska et al., 2006). In growing cattle, dietary supplements of a mixture (1:1 wt/wt) of FO and linseed oil (**LO**) lowered ruminal biohydrogenation of 20:5n-3 and 22:6n-3 and increased *trans*-11 18:1 at the duodenum compared with the same amount of oil from FO alone (Shingfield et al., 2011). These findings suggest that dietary supplements of a mixture of plant oils and FO could be used to increase ruminal escape of *trans*-11 18:1, 18-carbon PUFA, and long-chain n-3 FA for incorporation into meat and milk.

Changes in ruminal biohydrogenation to dietary FO supplements are accompanied by alterations in the ruminal bacterial community capable of biohydrogenation (Kim et al., 2008; Huws et al., 2010; Shingfield et al., 2012). Populations of specific bacteria in the rumen were found to be altered by marine algae supplements in sheep fed diets containing sunflower oil (**SO**; Toral et al., 2012), but no reports exist on the effects of FO with plant oils on the abundance of biohydrogenating bacteria in cattle. Characterizing the effects on ruminal biohydrogenation is central to understanding the mechanisms underpinning physiological responses to lipid supplements in lactating ruminants and the effect on milk fat composition, which is a major source of SFA in the human diet (Eilander et al., 2015).

The present experiment tested the hypotheses that (1) plant-derived 18-carbon PUFA may lower ruminal biohydrogenation of long-chain n-3 FA in FO and increase *trans*-11 18:1, (2) that the resultant accumulation of intermediates is associated with changes in the key bacterial species known to be capable of biohydrogenation, and (3) that the effects may differ depending on whether 18:2n-6 or 18:3n-3 is the main source of PUFA. The experimental treatments were formulated to meet the objectives of providing new information on changes in ruminal biohydrogenation of long-chain polyenoic FA, metabolic pathways involved, and specific FA products and intermediates associated with these diets.

MATERIALS AND METHODS

Cows and Experimental Design

All experimental procedures were approved by the Animal Experiment Committee of MTT Agrifood Research Finland in accordance with the guidelines established by the European Community Council Directive 86/609/EEC (European Council, 1986). Four multiparous Finnish Ayrshire cows (193 ± 16.2 d postpartum, 637 ± 22.3 kg of live weight, and producing 24.8 ± 4.73 kg of milk/d) fitted with rumen cannulas (i.d. 100 mm; Bar Diamond Inc., Parma, ID) were used in a 4 × 4 Latin square with 21-d experimental periods. Treatments comprised a basal diet containing no additional oil (control), or supplemented with 200 g of ultra refined herring and mackerel oil (EPAX 3000 TG Pronova Biocare AS, Aalesund, Norway; treatment **FO**), 200 g of FO and 500 g of SO (Raisioagro Ltd., Raisio, Finland; **SFO**) or 200 g of FO and 500 g of LO per day (Elixi Oil Ltd., Somero, Finland; **LFO**; Supplemental Table S1; https://doi.org/10.3168/jds.2017-13776). Cows were housed in individual tiestalls within a dedicated metabolism unit with continuous access to water and salt block and were milked daily at 0700 and 1645.

Experimental Diets

Cows were offered grass silage and a cereal-based concentrate (forage:concentrate ratio 60:40, on a DM basis) as equal meals at 0600 and 1800 at 95% of ad libitum intake measured during 14-d before the start of the experiment. Experimental silage was prepared from primary growths of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). The concentrate supplement contained (g/kg): rolled barley (520), molassed sugar beet pulp (250), solvent extracted rapeseed meal (200) of low glucosinolate content (Raisioagro Ltd., Raisio, Finland), and a mineral and vitamin premix (30; Onni-Kivennäinen, Rehumelica, Vaasa, Finland). Oil supplements were fed in equal amounts at 0600 and 1800 by mixing with concentrate ingredients immediately before feeding.

Sampling and Chemical Analysis

Intake of each cow was measured daily. Representative samples of feed ingredients and feed refusals were collected daily over the last 5 d of each experimental period for analysis of chemical composition (Shingfield et al., 2002). Samples of ruminal fluid (n = 8) were collected from each cow at 1.5-h intervals from 0600 to 1500 h on d 20 (0600, 0730, 0900, 1030, 1200, 1330, and 1500 h, respectively). Samples (approximately 500 mL) were collected through rumen cannula using a vacuum pump and flexible tube and analyzed for pH, VFA and ammonia N concentrations (Shingfield et al., 2002). An additional 10-mL subsample of filtered rumen fluid was diluted with 30 mL of 0.9% (wt/vol) aqueous NaCl