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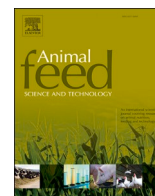
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## Energy utilization and milk fat responses to rapeseed oil when fed to lactating dairy cows receiving different dietary forage to concentrate ratio

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### ABSTRACT

We evaluated energy and N utilization, performance, and milk fatty acid (FA) profile using grass silage-based diets when rapeseed oil (RO) was included in high- or low-forage diets. Four multiparous Nordic Red cows averaging  $101 \pm 16$  days in milk at the beginning of the study were randomly assigned to a  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of treatments. Each 21-d period consisted of a 14-d diet adaptation period and 7-d collection period. Cows were fed the following diets comprised total mixed rations based on grass silage with forage to concentrate (FC) ratio of 35:65 and 65:35 containing 0 or 50 g/kg of RO. Significant FC  $\times$  RO interactions were observed for milk yield, milk protein and lactose yields, milk fat concentration, and milk proportions of *trans*-11 18:1, *trans*-10 18:1, *trans*-10, *cis*-12 18:2, and saturated FA. Feeding low-forage diet was effective in increasing milk yield compared with the high-forage diet, and the RO supplementation increased it further ( $P \leq 0.01$ ). A similar pattern was observed for the yields of milk protein and lactose. Supplementing the low-forage diet with RO reduced milk fat concentration by 19% relative to other diets without affecting milk fat yield. The proportion of N intake lost as urine decreased ( $P \leq 0.05$ ) with the RO supplementation of low-forage diet without affecting energy and N balances. Nutrient intakes were greater ( $P \leq 0.01$ ) in cows fed low-forage diet, whereas RO decreased ( $P < 0.05$ ) protein, starch, and fiber intakes. Methane production, expressed as a proportion of energy intake, decreased with low-forage compared with high-forage diets and this variable declined similarly by RO supplementation of both diets ( $P < 0.01$ ). The milk proportions of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA increased ( $P \leq 0.01$ ) by RO supplementation of the low-forage but not high-forage diet. However, RO supplementation of both high- and low-forage diets increased ( $P < 0.01$ ) total *trans* FA and decreased saturated FA proportions, even though the changes were more profound in low-forage diet ( $P \leq 0.01$ ). In addition, RO increased ( $P < 0.01$ ) *cis* monounsaturated FA in milk for both high- and low-forage diets. Overall, the low-forage diets had lower methane emissions and RO increased partitioning of

**Abbreviations:** BH, biohydrogenation; BW, body weight; CLA, conjugated linoleic acids; CP, crude protein; DE, digestible energy; DM, dry matter; DMI, DM intake; ECM, energy-corrected milk; FA, fatty acid; FAME, fatty acid methyl esters; FC ratio, forage-to-concentrate ratio; HF, high-forage diet; NDF, indigestible neutral detergent fiber; LF, low-forage diet; ME, metabolizable energy; MFD, milk fat depression; NDF, neutral detergent fiber expressed exclusive of residual ash; NFC, non-fibre carbohydrates; NRC, National Research Council; SD, standard deviation; TMR, total mixed ration; UFA, unsaturated fatty acid; VFA, volatile fatty acid.

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N towards milk secretion ( $P \leq 0.01$ ) without influencing energy or N balances. According to the results, RO supplementation did not compromise intake of nutrients with low-forage diets containing 150 g/kg starch, and oil could be preferentially used to improve milk production and milk fat quality accompanied by a reduction in methane energy loss.

## 1. Introduction

In dairy cow rations, modifying forage to concentrate (FC) ratio or supplementation with unsaturated fat affect energy and nitrogen (N) utilization and potentially mitigates enteric methane emissions from ruminants (Sterk et al., 2011; Nichols et al., 2019; Morris et al., 2020). Diet digestibility and energy utilization are partly related to dietary NDF concentration and NDF digestibility from forage sources (Huhtanen et al., 2009). Also, forage source, and dietary lipid and starch contents are known to affect energy and nitrogen (N) utilization in lactating dairy cows (Sterk et al., 2011; Nichols et al., 2019; Morris et al., 2020). Increasing dietary starch content rises energy supply in the rumen, which supports microbial growth and rumen outflow of microbial protein (Roman-Garcia et al., 2016; White et al., 2016) whereas lipids are modified extensively in the rumen and utilized mainly as post-absorptive energy sources, which may be directly transferred to milk fat (Harvatine et al., 2009; Boerman et al., 2015). Full-fat rapeseed and rapeseed oil, containing the greatest content of unsaturated fatty acid (UFA) especially oleic acid (*cis*-9 18:1) and linoleic acid (*cis*-9, *cis*-12 18:2), have been investigated for their effects on lactation performance (Kliem et al., 2019), fatty acid (FA) profile (Welter et al., 2016), and enteric methane emissions (Beauchemin et al., 2009) in dairy cows.

A high proportion of concentrate (Piperova et al., 2002), or a combination of dietary high proportion of concentrate and UFA (Griinari et al., 1998) have been outlined as dietary factors inducing milk fat depression (MFD; Bauman and Griinari, 2003). The lower milk fat concentration and yield often reduce energy demand for milk production in cows resulting in enhanced adipose de novo FA synthesis, which accounts for, at least in part, the increased tissue energy during MFD (Moore et al., 2004; Harvatine et al., 2009). Dietary FC ratio and plant oil supplements affect rumen fermentation pathways, resulting in different volatile fatty acid profile and thereby methane production (Beauchemin et al., 2020), which may alter energy or N partitioning (Aguerre et al., 2011; Bayat et al., 2017). With respect to differential effects of energy sources on microbial protein synthesis, production of fermentation acids in the rumen, and post-absorptive metabolism, they are expected to affect both milk production and components differently (Nichols et al., 2019; Morris et al., 2020). Furthermore, increasing energy supply from lipids may increase the efficiency at which ME is converted into milk energy through the direct transfer of dietary FA into milk (Hammon et al., 2008).

Energy from lipids and forage or starch may interact to affect N and energy partitioning and interactions likely exist between FC ratio and lipid supplement, but these interactions are not entirely understood. The main objective of the present study was to quantify the effects of high- and low-forage diets with or without rapeseed (*Brassica napus*) oil (RO) supplementation (i.e., providing simultaneous glucogenic and lipogenic nutrients) on milk production, energy and N metabolism, efficiency of energy utilization, and milk fat biohydrogenation (BH) intermediates of Nordic Red cows in mid-lactation. We hypothesized that supplement of RO would increase the energy utilization for milk production, especially when added to a low-forage diet, in addition to altering milk FA profile. Besides, we hypothesized that lower dietary FC ratio would have positive effects on N metabolism by dietary N use efficiency.

## 2. Materials and methods

This experiment was conducted at Luke's research barn, Jokioinen, Finland in 2019. Animal care and all experimental procedures used in this study were approved by the National Ethics Committee (ESAVI/24435/2018, Hämeenlinna, Finland) in accordance with the guidelines established by the European Community Council Directive 2010/63/EU (EU 2010) for animal experiments and complied with the ARRIVE guidelines.

**Table 1**  
Chemical composition of feed ingredients.

Item	Grass silage <sup>A</sup>	Concentrate (High forage diet)	Concentrate (Low forage diet)	Rapeseed oil
Dry matter, g/kg	266	877	875	1000
Organic matter, g/kg DM	942	914	931	1000
Crude protein, g/kg DM	132	191	195	0
Neutral detergent fiber, g/kg DM	549	271	276	0
Ether extract, g/kg DM	33.7	35.1	37.0	824.1
Starch, g/kg DM	13.8	219	232	0
Water soluble carbohydrates, g/kg DM	32.6	66.4	66.7	0
Gross energy, MJ/kg DM	17.9	18.2	18.7	39.0

<sup>A</sup>Mean fermentation characteristics of experimental silage: pH, 4.09; in DM (g/kg) lactic acid, 65.4; acetic acid, 18.0; propionic acid, 0.25; butyric acid, 1.03; soluble N (g/kg of total N), 597; ammonium N (g/kg of total N), 71.7.

## 2.1. Cows, experimental design, and treatments

Four multiparous Nordic Red dairy cows were assigned randomly to treatment sequences in a  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of treatments. Cows had (mean  $\pm$  SD)  $632 \pm 46$  kg body weight,  $3.5 \pm 0.58$  parity, and  $101 \pm 16$  days in milk with milk yield of  $38.8 \pm 1.9$  kg/d at the beginning of the experiment. Each experimental period lasted for 21 d consisting of 14 d for adaptation and 7 d for sample and data collection. Treatments comprised diets based on grass silage (FC ratio 65:35 or 35:65 on a DM basis) containing either no added oil [high-forage diet (HF) and low-forage diet (LF)] or 50 g/kg diet DM of RO [high-forage diet plus oil (HFO) and low-forage diet plus oil (LFO); Tables 1 and 2]. The semi-purified rapeseed oil (AvenaKantvik Ltd, Kirkkonummi, Finland) was stored in  $4^\circ\text{C}$  until incorporated into the low or high FC ratio diets and RO replaced concentrate ingredients. Treatments were supplemented with 50 g/kg DM of a UFA source (rapeseed oil) based on our previous experiments (Bayat et al., 2017, 2018) to induce maximum effects on milk FA and enteric methane production without compromising the DMI of the cows. The appropriate amount of rapeseed oil for each cow was weighed daily and mixed thoroughly with the TMR. The forage was restrictively fermented grass silage prepared from primary growth of mixed timothy (*Phleumpratense*) and meadow fescue (*Festuca pratensis*) swards, grown at Jokioinen ( $60^\circ 49' \text{N}$ ,  $23^\circ 28' \text{E}$ ) and treated at harvest with a formic acid-based ensiling additive (5 L/ton, AIV 2 Plus, Eastman/Taminco Finland Oy, Finland). Before ensiling in the bunker silo, the grass was wilted on the field to reach about 30% DM. Experimental diets were offered ad libitum to result in 10% refusals and formulated to meet requirements for ME and protein of lactating cows producing 40 kg milk/d (Finnish Feed Evaluation System; Luke, 2021). Diets contained grass silage (as the only forage source), rapeseed meal, rolled barley, ground oats, molassed sugar beet pulp, and a premix of minerals and vitamins. Chemical composition of grass silage, supplemental concentrates and rapeseed oil is presented in Table 1. The grass silage had a relatively high quality as indicated by its fermentation characteristics and chemical composition. The HF and LF concentrate pellets used to make dietary treatments with 65:35 and 35:65 FC ratios (on DM basis), respectively had similar formulation except vitamin and mineral premix which for LF was half of that in HF concentrate. This resulted in differences in diet energy and N contents due to differences in FC ratio between HF and LF groups (Table 2). The diets were prepared as TMR to avoid selection of dietary components (i.e., silage and concentrate pellets) and maintain the desired FC ratio, and offered daily at 0600, 0900, 1600, and 1900 h. Cows were housed in free stalls in the barn during adaptation period and in the respiration chambers during the sampling period with free access to water and salt block and were milked at 0700 and 1645 h in a  $2 \times 6$  auto tandem parlor milking system.

## 2.2. Measurements and chemical analysis

Daily feed intake and milk yield were recorded throughout the experiment, but only measurements made between day 15 and 21 of each experimental period were used for statistical analysis. During this period, representative samples of silage and concentrates were collected daily, composited, and stored frozen ( $-20^\circ\text{C}$ ) until submitted for chemical composition [dry matter (DM), ash, crude protein (CP), neutral detergent fiber (using sodium-sulfite and heat-stable  $\alpha$ -amylase and corrected for ash, NDF), ether extract (EE), starch,

**Table 2**  
Formulation and chemical composition of experimental total mixed ration.

Item	Treatments <sup>A</sup>			
	HF	HFO	LF	LFO
Feed ingredients, g/kg DM				
Grass silage	650	650	350	350
Rolled barley	78.8	67.5	150	138
Ground oats	78.8	67.5	150	138
Molassed sugar beet pulp	78.8	67.5	149	137
Rapeseed meal	101	86.4	190	176
Rapeseed oil <sup>B</sup>	–	50	–	50
Vitamin and mineral premix <sup>C</sup>	13.0	11.1	11.7	10.8
Chemical composition, g/kg DM unless stated				
Dry matter, g/kg as-fed	479	486	662	668
Organic matter	932	936	935	938
Crude protein	153	143	173	163
Neutral detergent fiber	452	438	372	358
Forage neutral detergent fiber	357	357	192	192
Potentially degradable NDF <sup>D</sup>	360	350	286	276
Ether extract	34.2	73.6	35.9	75.2
Starch	86.0	75.0	155	144
Water soluble carbohydrates	44.0	41.0	55.0	51.0
Gross energy, MJ/kg DM	18.0	19.1	18.4	19.4

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional lipid (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional lipid (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO).

<sup>B</sup>Rapeseed oil containing (g/100 g total fatty acids) 16:0 (4.06), 18:0 (1.85), *cis*-9–18:1 (58.4), *cis*-11–18:1 (3.38) and *cis*-9, *cis*-12–18:2 (19.5) as major components and gross energy content 41.4 MJ/kg of DM.

<sup>C</sup>Premix (Mahti-Mira, Vilomox Finland, Paimio) declared as containing (g/kg) Ca (220), Na (105), and Mg (64) and (mg/kg) vitamins A (156), D (52), E (1144), CuSO<sub>4</sub> (1180), MnSO<sub>4</sub> (1025), ZnO (2127), Ca (IO<sub>3</sub>)<sub>2</sub> (66), Na<sub>2</sub>SeO<sub>3</sub> (33), Se (37), and CoCO<sub>3</sub> (520).

<sup>D</sup>Potentially degradable NDF.

and water-soluble carbohydrates] determinations as described by Ahvenjärvi et al. (2018). Gross energy (GE) in samples of silage, concentrates, oil supplement, and excreta was determined by bomb calorimetry (1108 Oxygen bomb, Parr Instrument, Illinois). Indigestible NDF (iNDF) of silage, concentrate, and feces was determined by day 12 of ruminal incubation using nylon bags (60 × 120 mm, pore size 0.017 mm) followed by NDF analysis. Two fistulated dairy cows in mid-lactation fed grass silage-based diets (55:45 FC ratio on DM basis) were used for the iNDF determination. Dry matter content of silage was corrected for the loss of volatiles according to Huida et al. (1986).

Four open-circuit respiratory chambers (21.5 m<sup>3</sup>) were used to measure gas exchanges (oxygen, carbon dioxide, and methane) of the cows individually. The measuring system is described in detail elsewhere (Bayat et al., 2022). Briefly, concentrations of the gases in the inlet and exhaust airflow were measured by a computer-controlled system using dedicated analyzers (Oxymax, Columbus Instruments, Columbus, OH). Air outflow for each chamber was measured by HFM-200 mass flow meter with a laminar flow element capable of measuring up to 3000 L/min (Teledyne Hastings Instruments, Hampton, VA). Absolute gas exchanges were calculated by multiplying air flow and gas concentration differences under standard temperature and pressure conditions. Gas recovery tests were conducted before the experiments by releasing 3 levels of carbon dioxide, nitrogen (for diluting oxygen), and methane mimicking minimum, average and maximum gas exchanges by the cows based on our previous experiments. The recovery rates were 103.1 ± 5.1, 100.1 ± 0.04, and 100.7 ± 1.95% for carbon dioxide, oxygen, and methane, respectively.

Total-tract apparent digestibility coefficients were determined by total fecal collection over a 72-h interval starting at 1000 h on d 17 of each experimental period. Excreted feces were weighed, thoroughly mixed, subsampled (5%, wt/wt), and stored at -20 °C before chemical analysis. Urine was separated from feces by means of a lightweight harness and flexible tubing attached to the vulva, and collected in plastic canisters containing 500 mL of 5 M sulfuric acid. Collection vessels were changed at 12-h intervals and daily samples (5%, wt/wt) were taken and stored at -20 °C. Milk samples were taken for 3 consecutive days from d 17 (evening) to 20 (morning) of each period, treated with a preservative (Bronopol, Valio Ltd., Helsinki, Finland) and stored at 4 °C for analysis of milk fat, crude protein, and lactose by infrared analysis (MilkoScan 133B, Foss Electric, Hillerød, Denmark). Daily milk composition was calculated according to morning and evening milk yields. For analysis of milk FA composition using GC, milk was sampled during d 18 and 19 (evening and morning, respectively), pooled according to milk yield, and stored at -20 °C without preservative.

### 2.3. Lipid analysis

Fatty acid methyl esters of lipid in feed and milk samples were prepared as described by Shingfield et al. (2003). Briefly, fat in 1 mL of milk was extracted twice with a mixture of ammonia, ethanol, diethyl ether, and hexane (0.2:1:2.5:2.5, vol/vol). Fatty acids were then methylated with methanolic sodium methoxide in the presence of methyl acetate. Total FAME profile was determined using a GC (6890 N, Agilent Technologies, Santa Clara, CA) equipped with a CP-Sil 88 column (100 m × 0.25 mm i.d., 0.2 µm film thickness, Agilent Technologies) and flame ionization detector with a temperature gradient program (Shingfield et al., 2003). Identification was based on retention time comparisons with authentic FAME standards (Larodan Fine Chemicals AB, Malmö, Sweden; Nu-Chek Prep Inc., Elysian, MN; Sigma-Aldrich) and previous milk samples verified by GC-MS (6890 and 5973, Agilent Technologies) and silver ion HPLC (Shingfield et al., 2003). Hydrogen was used as a carrier gas starting from 206.8 kPa initial pressure and gradually raised after 50 min to a final pressure of 310.3 kPa.

### 2.4. Calculations and statistical analysis

Potentially digestible NDF was calculated as NDF - iNDF. Energy intake and energy excretion in feces and urine were calculated by multiplying DMI or feces and urine DM by their respective GE contents. Energy-corrected milk was calculated as ECM = milk (kg/d) × [38.3 × fat (g/kg) + 24.2 × crude protein (g/kg) + 16.54 × lactose (g/kg) + 20.7]/3140; Sjaunja et al. (1990)). Energy secretion in milk was calculated by multiplying ECM by 3.14. Heat production of the cows was calculated based on the exchanged oxygen, carbon dioxide, and methane and urinary N excretion measured in the respiratory chambers using the following equation: Heat Production (MJ/d) = 16.18 × O<sub>2</sub> + 5.16 × CO<sub>2</sub> - 5.90 × urinary N - 2.42 × CH<sub>4</sub> where the gases are expressed in liters per day and urinary N is expressed in gram per day (Brouwer, 1965). Methane energy was calculated using the conversion factor of 55.24 kJ for every gram of methane (Kriss et al., 1930). Energy balance was calculated as the difference between energy intake and energy excretion as feces, urine, methane, milk, and heat. Daily ME intake was calculated as the difference between GE intake and energy excretions as feces, urine, and methane. Nitrogen balance was calculated as the difference between N intake and excretion in feces, urine, and milk.

Before statistical analysis, all data were tested for normality of distribution using Proc Mixed (version 9.4, SAS Institute Inc., Cary, NC). The DM intake and the yield of milk and milk components were averaged before statistical analysis. Measurements of intake, energy and N utilization, milk production, and milk FA composition were analyzed by ANOVA for a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments, with a statistical model that included the fixed effects of period, FC ratio, RO supplementation and their interaction, and the random effect of cow using the PROC MIXED procedure of SAS. Least-square means with their standard errors are reported and the effects of FC ratio, RO, and their interaction were declared significant at  $P \leq 0.05$ . Probabilities at  $0.05 \leq P \leq 0.10$  were considered as a trend. The means were compared together using Tukey test when the interaction was significant.

### 3. Results

#### 3.1. Nutrient composition of diets

The ingredient and chemical composition of the experimental diets are reported in Table 2. Experimental diets contained different concentrations of CP (148 vs. 168 g/kg DM) and GE(18.5 vs. 18.9 MJ/kg DM) for HF than LF diets, respectively. As expected, the EE concentration was greater (74.4 vs. 35.0 g/kg DM) for diets supplemented with RO than those without RO, and the starch concentration was greater for LF than HF diets (149 vs. 81 g/kg DM basis); as a consequence, fiber fractions exhibited the opposite trend due to changes in barley and oats proportions. The variation in dietary FA was mostly driven by the replacement of barley and oats with RO and altering dietary FC ratio. Silage had high quality, in terms of nutritive value and fermentation characteristics.

#### 3.2. Nutrient intakes and lactation performance

Dry matter intake increased by 8% ( $P < 0.05$ ) for LF than HF diets (Table 3). Intakes of OM, CP, and starch were greater ( $P \leq 0.01$ ), and that of NDF and pdNDF were lower ( $P < 0.01$ ) for LF than HF diets. Supplementing RO tended to decrease ( $P = 0.09$ ) DMI for HF diet. In addition, RO led to lower ( $P < 0.05$ ) intakes of CP, starch, and NDF. Further, EE intake increased ( $P < 0.01$ ) by supplementing RO whereas GE intake was not affected. Dietary FA intakes were significantly affected by the interaction between RO and FC ratio ( $P \leq 0.05$ ); in particular, intake of *cis*-9 18:1, *cis*-9, *cis*-12 18:2, and *cis*-9, *cis*-12, *cis*-15 18:3 increased in LFO. Also, intake of total FA was greater ( $P < 0.01$ ) with RO-supplemented diets compared with the diets without the supplement, specifically, cows fed LFO diet had greatest daily FA intake than the rest of the cows ( $P < 0.05$  for interaction of FC and RO).

The LF diet increased yields of milk, milk protein, and lactose compared with HF, and RO increased them only when supplemented to LF diet ( $P < 0.05$  for FC  $\times$  RO interaction). In addition, milk fat concentration decreased in LFO diet ( $P = 0.026$  for FC  $\times$  RO interaction). The yields of ECM ( $P < 0.01$ ) and milk fat ( $P < 0.05$ ), and concentration of milk protein ( $P < 0.05$ ) were greater for LF than HF diets (Table 4). Feed efficiency, calculated as kg of milk per kg of DMI, improved ( $P < 0.01$ ) by feeding LF diet to dairy cows compared with HF and by supplementing RO, similar pattern was observed ( $P = 0.017$ ). However, treatments had no effect ( $P \geq 0.12$ ) on the feed efficiency calculated as ECM/DMI.

**Table 3**

Effect of dietary forage to concentrate ratio and rapeseed oil on nutrient and fatty acids intake of lactating cows fed grass silage-based diets.

Item	Treatments <sup>A</sup>				SEM	P-value <sup>B</sup>		
	HF	HFO	LF	LFO		FC	RO	FC $\times$ RO
Intake, kg/d								
Dry matter	24.7	22.3	25.8	25.6	0.83	0.015	0.091	0.13
Organic matter	23.1	20.8	24.1	24.1	0.78	0.014	0.11	0.13
Crude protein	3.76	3.20	4.43	4.17	0.120	< 0.01	< 0.01	0.18
Neutral detergent fiber <sup>C</sup>	11.3	9.83	9.73	9.31	0.389	0.013	0.020	0.13
Forage NDF	9.01	8.05	5.22	5.17	0.310	< 0.01	0.084	0.11
pdNDF <sup>D</sup>	8.99	7.86	7.50	7.20	0.312	< 0.01	0.023	0.13
Ether extract	0.85 <sup>c</sup>	1.56 <sup>b</sup>	0.92 <sup>c</sup>	1.87 <sup>a</sup>	0.048	< 0.01	< 0.01	0.022
Starch	2.06	1.64	3.91	3.60	0.082	< 0.01	< 0.01	0.50
Gross energy, MJ/d	447	423	475	497	15.8	< 0.01	0.93	0.12
Fatty acid, g/d								
14:0	2.59 <sup>b</sup>	2.81 <sup>ab</sup>	2.32 <sup>c</sup>	2.90 <sup>a</sup>	0.101	0.28	< 0.01	0.052
16:0	104 <sup>c</sup>	127 <sup>b</sup>	121 <sup>b</sup>	160 <sup>a</sup>	4.9	< 0.01	< 0.01	0.052
18:0	8.17 <sup>c</sup>	24.8 <sup>b</sup>	9.38 <sup>c</sup>	30.2 <sup>a</sup>	0.81	< 0.01	< 0.01	0.010
<i>cis</i> -9 18:1	87.8 <sup>d</sup>	631 <sup>b</sup>	155 <sup>c</sup>	813 <sup>a</sup>	20.6	< 0.01	< 0.01	< 0.01
<i>cis</i> -11 18:1	14.4 <sup>d</sup>	43.6 <sup>b</sup>	25.5 <sup>c</sup>	61.5 <sup>a</sup>	1.43	< 0.01	< 0.01	0.014
<i>cis</i> -9, <i>cis</i> -12 18:2	171 <sup>d</sup>	321 <sup>b</sup>	252 <sup>c</sup>	448 <sup>a</sup>	11.7	< 0.01	< 0.01	0.035
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	164 <sup>c</sup>	231 <sup>b</sup>	109 <sup>d</sup>	209 <sup>a</sup>	7.9	< 0.01	< 0.01	0.024
20:0	5.27 <sup>b</sup>	10.0 <sup>a</sup>	3.87 <sup>c</sup>	10.2 <sup>a</sup>	0.32	0.055	< 0.01	0.031
<i>cis</i> -11 20:1	2.19 <sup>d</sup>	11.9 <sup>b</sup>	3.60 <sup>c</sup>	15.5 <sup>a</sup>	0.39	< 0.01	< 0.01	0.010
$\Sigma$ SFA	151 <sup>c</sup>	198 <sup>b</sup>	159 <sup>c</sup>	233 <sup>a</sup>	7.2	0.010	< 0.01	0.036
$\Sigma$ MUFA	123 <sup>d</sup>	716 <sup>b</sup>	203 <sup>c</sup>	925 <sup>a</sup>	23.3	< 0.01	< 0.01	0.010
$\Sigma$ PUFA	338 <sup>c</sup>	564 <sup>b</sup>	365 <sup>c</sup>	675 <sup>a</sup>	19.7	< 0.01	< 0.01	0.023
$\Sigma$ Fatty acids	615 <sup>c</sup>	1484 <sup>b</sup>	728.6 <sup>c</sup>	1841 <sup>a</sup>	49.7	< 0.01	< 0.01	0.013

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional oil (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional oil (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO). Values are LS means and pooled SEM for  $n = 4$ .

<sup>B</sup>FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC  $\times$  RO, interaction of forage to concentrate ratio and rapeseed oil supplement.

<sup>C</sup>aNDFom

<sup>D</sup>Potentially digestible NDF.

**Table 4**

Effect of dietary forage to concentrate ratio and rapeseed oil on milk yield and milk composition of lactating cows fed grass silage-based diets.

Item	Treatments <sup>A</sup>				SEM	P-value <sup>B</sup>		
	HF	HFO	LF	LFO		FC	RO	FC × RO
Yield, kg/d								
Milk	31.2 <sup>c</sup>	30.5 <sup>c</sup>	36.2 <sup>b</sup>	40.6 <sup>a</sup>	1.47	< 0.01	0.038	0.011
ECM <sup>C</sup>	33.3	32.9	39.4	39.4	1.95	< 0.01	0.82	0.82
Fat	1.43	1.40	1.64	1.49	0.095	0.028	0.24	0.22
Protein	1.12 <sup>c</sup>	1.08 <sup>c</sup>	1.37 <sup>b</sup>	1.46 <sup>a</sup>	0.070	< 0.01	0.26	0.020
Lactose	1.40 <sup>c</sup>	1.37 <sup>c</sup>	1.63 <sup>b</sup>	1.85 <sup>a</sup>	0.080	< 0.01	0.040	0.014
Concentration, g/kg								
Fat	44.9 <sup>a</sup>	45.8 <sup>a</sup>	45.2 <sup>a</sup>	36.6 <sup>b</sup>	1.79	0.032	0.054	0.026
Protein	36.2	35.6	37.8	36.1	1.64	0.036	0.020	0.20
Lactose	45.0	45.0	45.1	45.5	4.3	0.13	0.090	0.16
Urea, mg/dL	23.9	19.2	30.3	22.7	1.97	< 0.01	< 0.01	0.22
Feed efficiency								
Milk/DMI	1.26	1.38	1.41	1.58	0.045	< 0.01	0.017	0.56
ECM/DMI	1.35	1.48	1.53	1.53	0.067	0.12	0.33	0.35

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional lipid (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional lipid (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO). Values are LS means and pooled SEM for n = 4.

<sup>B</sup>FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC × RO, interaction of forage to concentrate ratio and rapeseed oil supplement.

<sup>C</sup>Energy-corrected milk calculated as described by Sjaunja et al. (1990).

### 3.3. Energy and nitrogen metabolism

The effects of diets on variables related to energy utilization and excretion are reported in Table 5. Dietary treatments had no effect ( $P \geq 0.22$ ) on milk energy secretion as a proportion of either GE or ME intake, urine energy excretion as a proportion of GE intake, heat production, and energy balance. Feeding LF diets increased ( $P < 0.01$ ) GE, DE and ME intakes and energy metabolizability (ME to GE intake) while it decreased feces ( $P < 0.05$ ) and methane ( $P < 0.01$ ) energy excretion as a proportion of GE intake compared with HF diets. Further, supplementing diets with RO caused a decrease ( $P < 0.01$ ) in methane energy excretion as a proportion of GE intake compared with un-supplemented diets. Daily methane emission was lowered by RO supplementation and the response was stronger with HF diet ( $P < 0.05$  for the interaction of FC and RO). Methane yield was lowered ( $P < 0.01$ ) by both greater concentrate ratio and dietary RO supplement while methane intensity tended to be reduced by RO more profoundly when supplemented to HF than LF diet

**Table 5**

Effect of dietary forage to concentrate ratio and rapeseed oil on energy and N metabolism, methane and carbon dioxide emissions, and oxygen consumption of lactating cows fed grass silage-based diets.

Item <sup>C</sup>	Treatments <sup>A</sup>				SEM	P-value <sup>B</sup>		
	HF	HFO	LF	LFO		FC	RO	FC × RO
Energy								
Gross energy intake, MJ/d	447	423	475	497	15.8	< 0.01	0.93	0.12
DE intake, MJ/d	288	265	316	326	11.3	0.003	0.49	0.13
ME intake, MJ/d	244	227	272	285	9.7	0.003	0.83	0.14
Proportion of energy intake, kJ/MJ								
Feces	354	374	335	344	8.5	0.016	0.12	0.54
Urine	32.0	32.7	32.0	29.7	1.60	0.25	0.31	0.24
Methane	66.1	56.0	60.0	52.2	2.40	< 0.01	< 0.01	0.26
Milk	234	245	261	249	11.4	0.22	0.94	0.34
Heat	294	296	299	285	9.3	0.56	0.29	0.17
Milk energy/ME intake	429	456	457	433	22.5	0.92	0.93	0.29
ME intake/GE intake	547	537	573	574	7.8	< 0.01	0.61	0.47
Energy balance, MJ/d	8.24	-1.40	6.63	19.9	10.31	0.32	0.85	0.25
Methane								
Emission, g/d	535 <sup>a</sup>	428 <sup>c</sup>	516 <sup>a</sup>	470 <sup>b</sup>	27.7	0.25	< 0.01	0.015
Yield, g/kg DMI	21.6	19.2	20.0	18.3	0.81	< 0.01	< 0.01	0.33
Intensity, g/kg ECM	16.0	13.0	13.2	12.0	0.64	< 0.01	< 0.01	0.087
O <sub>2</sub> consumption, g/d	8612	8291	9258	9320	467	< 0.01	0.29	0.14
CO <sub>2</sub> production, g/d	14,374	13,133	15,628	15,203	711	< 0.01	< 0.01	0.11

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional lipid (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional lipid (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO). Values are LS means and pooled SEM for n = 4.

<sup>B</sup>FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC × RO, interaction of forage to concentrate ratio and rapeseed oil supplement.

<sup>C</sup>DE, digestible energy; ME, metabolizable energy; GE, gross energy.

( $P = 0.09$  for the interaction of FC and RO). Both oxygen consumption and carbon dioxide production were lower ( $P < 0.01$ ) with LF compared with HF diets and RO reduced ( $P < 0.01$ ) carbon dioxide production but not oxygen consumption.

Supplement of RO resulted in decreased urine N as a proportion of N intake when included in LF, but not HF diet ( $P = 0.05$  for FC  $\times$  RO interaction; Table 6). Dietary treatments had no effect on N balance while feeding greater amount of concentrates increased ( $P < 0.01$ ) N intake but decreased ( $P < 0.05$ ) fecal N as a proportion of N intake compared with feeding lower amount of concentrates. However, supplementing diets with RO decreased ( $P = 0.01$ ) N intake and increased ( $P < 0.05$ ) N secreted in milk as a proportion of N intake.

### 3.4. Milk fatty acid composition

The effects of dietary treatments on milk FA profile are reported in Table 7. The RO supplement resulted in greater decreases in 8:0, 10:0, and 12:0 concentrations in milk fat when included in LF than HF diets ( $P < 0.01$  for FC  $\times$  RO interaction). A tendency was found for the interaction ( $P < 0.10$ ) between inclusion of RO and FC ratio on 4:0, 14:0 and *trans*-9 14:1 concentrations in milk fat; in particular, 4:0 and *trans*-9 14:1 decreased in HFO diet. Rapeseed oil supplementation increased the milk proportion of 18:0 in both LF and HF diets with more pronounced increase in HFO diet ( $P = 0.07$  for FC  $\times$  RO interaction). Dietary inclusion of RO increased the concentration of *trans*-11 18:1 more in cows fed HF diets, than those fed LF diets ( $P < 0.05$  for FC  $\times$  RO interaction). Furthermore, RO increased concentrations of *trans*-10 18:1 and *trans*-10, *cis*-12 18:2 in milk fat when included in LF diet but did not change the proportion of these FA in milk fat when included in HF diet ( $P \leq 0.01$  for FC  $\times$  RO interaction). *Trans*-10 18:1 was the major 18:1 intermediate for LFO treatment, whereas *trans*-11 18:1 was the most abundant 18:1 intermediate for HFO diet. Milk fat *trans* 18:1, *trans* 18:2, and total *trans*-FA concentrations were greater ( $P < 0.01$  for FC  $\times$  RO interaction) with LFO whereas SFA proportion was lower ( $P < 0.05$  for FC  $\times$  RO interaction). In addition, decreasing FC ratio increased ( $P < 0.01$ ) n-6/n-3 ratio in milk fat. Rapeseed oil decreased ( $P < 0.01$ ) the concentrations of 16:0, *cis* 16:1, total 16:1, *cis*-9, *cis*-12 18:2, *cis* 18:2, and *cis* PUFA and increased ( $P < 0.01$ ) the proportions of *trans*-16:1, *cis*-9 18:1, *cis*-9, *trans*-11 CLA, total CLA, *cis* MUFA, and *cis* UFA in milk fat.

## 4. Discussion

Our aim was to evaluate the effect of typical Nordic grass silage-based diets differing in lipogenic or glucogenic nutrients and UFA source on energy and N partitioning. To achieve this, we did not simply replace one ingredient for another but the whole concentrate ingredients replaced for the grass silage (except vitamin and mineral premix) which resulted in differences in diet energy and N contents due to the differences in forage and concentrate intakes. Two FC ratios (anisonitrogenic and anisoenergetic) were obtained by replacing grass silage with grains and sugar beet pulp. Based on how the diets were formulated, we expected to cause a greater increase in milk production, without compromising feed intake and milk components, when RO was supplemented to low- (moderate starch concentration) compared with high-forage (low starch concentration) diets.

### 4.1. Nutrient intakes

In our study, the HF and LF diets differed mainly in their starch, NDF, and CP contents. Consequently, the feed intake difference originated primarily from different composition of the carbohydrate fractions i.e., NDF content. The dietary NDF content was 445 and 365 g/kg on DM basis for HF and LF groups, corresponding to NDF intakes of 1.7% and 1.5% of BW, respectively. Multiple mechanisms regulate DMI of ruminants, but DMI generally increases with reducing diet NDF, especially dietary forage NDF content (Allen, 2000). The higher DMI of cows fed LF diets is consistent with the previous studies that evaluated alfalfa silage-based diets with 35:65 and 60:40 FC ratios (Yang and Beauchemin, 2007) or grass silage-based diets with 30:70 and 70:30 FC ratios (Saliba et al., 2014). Similarly, Sterk et al. (2011) reported that shifting from a high-forage to a low-forage (65:35 vs. 35:65 FC ratio) diet increased DMI and milk yield in dairy cows. This result could be related to dietary NDF concentration and longer retention time of forage particles restricting the flow of digesta through the gastrointestinal tract (Allen, 2000). Moreover, cows fed LF diets had a greater dietary protein intake

**Table 6**

Effect of dietary forage to concentrate ratio and rapeseed oil on nitrogen metabolism of lactating cows fed grass silage-based diets.

Item	Treatments <sup>A</sup>				SEM	P-value <sup>B</sup>		
	HF	HFO	LF	LFO		FC	RO	FC $\times$ RO
Nitrogen intake, g/d	601	511	709	667	19.2	< 0.01	< 0.01	0.18
Proportion of nitrogen intake, g/kg								
Milk	293	334	304	343	15.3	0.41	0.012	0.98
Feces	365	358	345	342	6.7	0.029	0.51	0.92
Urine	334 <sup>ab</sup>	341 <sup>ab</sup>	347 <sup>a</sup>	322 <sup>b</sup>	16.3	0.67	0.22	0.052
Nitrogen balance, g/d	5.19	-15.0	3.61	-3.66	9.148	0.61	0.18	0.50

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional lipid (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional lipid (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO). Values are LS means and pooled SEM for  $n = 4$ .

<sup>B</sup>FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC  $\times$  RO, interaction of forage to concentrate ratio and rapeseed oil supplement.



Table 7

Effect of dietary forage to concentrate ratio and rapeseed oil on fatty acid profile in milk of lactating cows fed grass silage-based diets.

FA <sup>C</sup> , g/100 g of total FA	Treatments <sup>A</sup>					SEM	P-value <sup>B</sup>		
	HF	HFO	LF	LFO	FC		RO	FC × RO	
4:0	4.03	3.82	3.81	3.24	0.157	< 0.01	0.005	0.083	
8:0	1.40 <sup>b</sup>	0.99 <sup>c</sup>	1.73 <sup>a</sup>	0.93 <sup>d</sup>	0.038	< 0.01	< 0.01	< 0.01	
10:0	3.04 <sup>b</sup>	1.92 <sup>d</sup>	4.19 <sup>a</sup>	1.94 <sup>c</sup>	0.086	< 0.01	< 0.01	< 0.01	
12:0	3.40 <sup>b</sup>	2.08 <sup>c</sup>	4.85 <sup>a</sup>	2.29 <sup>c</sup>	0.098	< 0.01	< 0.01	< 0.01	
14:0	12.4	9.1	13.2	9.2	0.17	0.017	< 0.01	0.061	
<i>trans</i> -9 14:1	0.016	0.011	0.016	0.015	0.0020	0.057	0.017	0.078	
<i>cis</i> -9 14:1	1.00 <sup>b</sup>	0.61 <sup>c</sup>	1.02 <sup>a</sup>	0.88 <sup>ab</sup>	0.080	< 0.01	< 0.01	0.017	
16:0	34.1	20.3	30.0	18.0	0.92	0.010	< 0.01	0.34	
Σ <i>cis</i> 16:1	1.99	1.46	1.94	1.49	0.118	0.79	< 0.01	0.36	
Σ <i>trans</i> 16:1	0.115	0.218	0.121	0.264	0.0113	0.047	< 0.01	0.105	
Σ 16:1	2.11	1.68	2.06	1.75	0.117	0.69	< 0.01	0.17	
18:0	9.4	18.0	9.2	15.4	1.06	0.045	< 0.01	0.069	
<i>cis</i> -9 18:1 <sup>D</sup>	16.5	25.3	15.1	26.5	0.92	0.53	< 0.01	0.24	
Σ <i>cis</i> 18:1	16.4	26.7	16.2	28.2	0.94	0.34	< 0.01	0.20	
<i>trans</i> -11 18:1	0.646 <sup>b</sup>	1.778 <sup>a</sup>	0.607 <sup>b</sup>	0.976 <sup>b</sup>	0.1200	< 0.01	< 0.01	0.014	
<i>trans</i> -10 18:1	0.189 <sup>b</sup>	0.513 <sup>b</sup>	0.290 <sup>b</sup>	3.249 <sup>a</sup>	0.1594	< 0.01	< 0.01	< 0.01	
Σ <i>trans</i> 18:1	1.83 <sup>c</sup>	5.03 <sup>b</sup>	2.03 <sup>c</sup>	7.68 <sup>a</sup>	0.232	< 0.01	< 0.01	< 0.01	
Σ 18:1	18.3 <sup>c</sup>	31.7 <sup>b</sup>	18.2 <sup>c</sup>	35.9 <sup>a</sup>	0.88	0.016	< 0.01	0.015	
<i>cis</i> -9, <i>cis</i> -12 18:2 <sup>E</sup>	1.12	0.90	1.63	1.44	0.045	< 0.01	< 0.01	0.52	
Σ <i>cis</i> 18:2	1.16	0.97	1.67	1.52	0.046	< 0.01	< 0.01	0.50	
Σ <i>trans</i> 18:2	0.913 <sup>c</sup>	1.648 <sup>b</sup>	0.886 <sup>c</sup>	2.015 <sup>a</sup>	0.1076	0.063	< 0.01	0.038	
Σ 18:2	2.07 <sup>c</sup>	2.62 <sup>b</sup>	2.56 <sup>b</sup>	3.53 <sup>a</sup>	0.145	< 0.01	< 0.01	0.045	
<i>cis</i> -9, <i>trans</i> -11 18:2 <sup>F</sup>	0.250	0.675	0.263	0.602	0.0623	0.59	< 0.01	0.45	
<i>trans</i> -10, <i>cis</i> -12 18:2	0.001 <sup>b</sup>	0.001 <sup>b</sup>	0.002 <sup>b</sup>	0.007 <sup>a</sup>	0.0008	< 0.01	0.061	0.013	
Σ CLA	0.338	0.748	0.352	0.707	0.0579	0.77	< 0.01	0.54	
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3 <sup>G</sup>	0.402 <sup>a</sup>	0.295 <sup>b</sup>	0.346 <sup>ab</sup>	0.340 <sup>ab</sup>	0.0255	0.80	0.031	0.049	
20:0	0.147	0.286	0.125	0.227	0.0168	0.025	< 0.01	0.22	
Σ <i>cis</i> 20:1	0.220	0.305	0.224	0.377	0.0239	0.13	< 0.01	0.16	
Σ <i>trans</i> 20:1	0.021	0.068	0.027	0.083	0.0033	0.010	< 0.01	0.16	
Σ 20:1	0.241	0.372	0.251	0.460	0.0259	0.073	< 0.01	0.13	
Σ Unidentified	0.105	0.087	0.095	0.081	0.0119	0.41	0.14	0.83	
Σ <i>cis</i> MUFA	20.6	29.7	20.4	31.6	1.05	0.24	< 0.01	0.15	
Σ <i>cis</i> PUFA	1.97	1.56	2.51	2.16	0.060	< 0.01	< 0.01	0.42	
Σ <i>cis</i> UFA	22.6	31.3	22.9	33.7	1.10	0.077	< 0.01	0.15	
Σ SFA	74.2 <sup>a</sup>	61.5 <sup>b</sup>	73.6 <sup>a</sup>	56.0 <sup>c</sup>	1.12	< 0.01	< 0.01	0.013	
Σ <i>trans</i> FA	3.11 <sup>c</sup>	7.13 <sup>b</sup>	3.35 <sup>c</sup>	10.2 <sup>a</sup>	0.28	< 0.01	< 0.01	< 0.01	
Σ <i>trans</i> FA + SFA	77.3	68.7	77.0	66.2	1.10	0.078	< 0.01	0.15	
Σ OBCFA	4.14	3.18	4.16	3.10	0.131	0.76	< 0.01	0.65	
Ratio									
<i>cis</i> -9 14:1/( <i>cis</i> -9 14:1 + 14:0)	0.075 <sup>b</sup>	0.063 <sup>c</sup>	0.072 <sup>bc</sup>	0.086 <sup>a</sup>	0.0068	0.019	0.78	< 0.01	
<i>cis</i> -9 18:1/( <i>cis</i> -9 18:1 + 18:0)	0.623 <sup>a</sup>	0.584 <sup>b</sup>	0.622 <sup>a</sup>	0.635 <sup>a</sup>	0.0238	0.004	0.050	< 0.01	
n-6/n-3 FA	2.12	2.20	3.35	3.25	0.110	< 0.001	0.92	0.44	

<sup>A</sup>Refers to diets with forage to concentrate (FC) ratio 65:35 with no additional lipid (HF); FC ratio 65:35 containing 50 g/kg diet DM of rapeseed oil (HFO); FC ratio 35:65 with no additional lipid (LF); and FC ratio 35:65 containing 50 g/kg diet DM of rapeseed oil (LFO). Values are LS means and pooled SEM for n = 4.

<sup>B</sup>FC, effect of forage to concentrate ratio in the diet; RO, effect of rapeseed oil supplement; FC × RO, interaction of forage to concentrate ratio and rapeseed oil supplement.

<sup>C</sup>FA, fatty acid; CLA, conjugated linoleic acid; OBCFA, odd- and branched-chain FA.

<sup>D</sup>Co-elutes with *trans*-13 18:1 and *trans*-15 18:1.

<sup>E</sup>Co-elutes with *cis*-9, *cis*-15 18:2 and *cis*-9 19:1

<sup>F</sup>Co-elutes with an unidentified FA.

<sup>G</sup>Co-elutes with *trans*-15 20:1 and *trans*-16 20:1.

(approximately 0.82 kg per day) than those fed HF diets and this difference can improve feed intake (M'Hamed et al., 2001).

We also observed a tendency for negative effect of RO supplementation on feed intake especially for cows fed HFO, even though interaction effect failed to reach statistical significance. The effect of oils in various forms (oil, oilseed cake, or crushed oilseeds) on feed intake of dairy cows has been generally negative (Glasser et al., 2008; Beauchemin et al., 2009). A concentration of > 500 g/d from sunflower oil or linseed oil tended to reduce DMI (Shingfield et al., 2008; Saliba et al., 2014) when included in the high-, but not low-concentrate diet. Despite lower feed intake in HFO relative to HF, they had similar GE intake, while no effect on nutrients intake was observed by supplementing RO to LF diet except for EE intake. Moreover, results from Benchaar et al., (2012, 2015) indicate that using vegetable oil at rates ranging from 3.5% to 5.5%, reduces intake and DM digestibility in cereal forage (e.g., corn silage) but not in high fiber based-diets (e.g., legume or grass forages). Therefore, it appears that the effect of diet energy density and energy intake from the oil supplement is different in diets containing various levels of starch, crude protein, and due to forage type. In addition, dietary addition of RO increased the intake of UFA (i.e., *cis*-9 18:1, *cis*-9, *cis*-12 18:2, and *cis*-9, *cis*-12, *cis*-15 18:3), and this increase led to a

greater magnitude with LF compared with HF.

#### 4.2. Production responses

It should be noted that due to the small number of cows and the relatively short experimental periods, the present study was not designed to measure production responses and results should be interpreted cautiously. Therefore, only production variables with significant effects are discussed herein.

The observed increase in milk yield with LF compared with HF diets could be explained by a greater intake of DM having higher nutrient density i.e., concentrates. The significant interaction between FC and RO on milk yield, reflected as the highest milk yield for LFO diet compared with other diets, is likely a response to difference in GE supply with dietary starch and oil. In our study, energy density and total GE intake were greater for LF diets; hence more energy was available in form of glucogenic or both glucogenic and lipogenic for milk production especially when RO was included in LF diet, with no compromise in feed intake. In an experiment in which 50 g/kg DM of sunflower oil was supplemented to a starch-rich diet (>300 g/kg of DM), feed intake tended to decrease (Ventto et al., 2017) without influencing milk production.

Although milk fat concentration decreased with LFO compared with the other 3 diets, milk fat yield was not different. Thus, decreased milk fat concentration could have originated from a dilution effect due to an increase in milk yield (~8 kg/d) with feeding LFO versus other diets. However, ECM yield was higher with LF compared with HF diets, regardless of RO supplementation. We observed that LFO diet decreased milk fat concentration by 19% whereas increased the concentration of *trans*-10 18:1, a good indicator for BH-induced MFD, in milk fat by 89% relative to other diets. However, the change in milk fat yield of LFO cows in the current study is not a typical MFD condition i.e., remarkable reduction in milk fat yield with no changes in milk yield and other milk components as proposed by Bauman and Griinari (2003), while cows having a relatively high milk fat content (37–46 g/kg). Although the concentration of *trans*-10 18:1 FA in milk fat from LFO was much greater than the mean suggested by Matamoros et al. (2020) to induce MFD (3.25 vs. 1.39 as percentage of FA), the cows had similar milk fat yield compared with other diets. As *trans* intermediates (i.e., *trans*-10 and *trans*-10, *cis*-12 CLA) are not the only regulating factors for milk fat yield, it appears that the greater feed intake with LF diets provides enough substrates for more milk fat synthesis. In contrast, it seems that the high dietary NDF concentration (on average 445 g/kg DM) in HF diets prevented decreases in milk fat concentration and yield when RO was included in the diet at 50 g/kg DM as suggested by Kliem et al. (2019). However, Saliba et al. (2014) compared diets differing in FC ratios (30:70 and 70:30) plus linseed oil supplement, which were different in dietary energy content, and observed that adding 30 g/kg linseed oil to both diets containing 120 and 366 g/kg starch decreased milk fat concentration. Further, Benchaar et al. (2015) indicated that diets containing mainly cereal forages (e.g., corn silage) are more sensitive to the depressive effects of vegetable oil on ruminal degradation of NDF and milk fat content than diets based on legume or grass silages. This result might be attributed to the forage source used and replacing strategy compared with our study (corn silage vs. grass silage). In lactating cows, the efficacy of linseed oil for reducing methane emissions was reported to be more profound when included in diets based on corn silage than red clover silage (Benchaar et al., 2015). In contrast, decreases in methane emissions following feeding of extruded linseeds were found to be similar in cows fed diets based on grass hay or corn silage (Martin et al., 2016).

Yield of milk protein increased in cows fed LF compared with HF while it increased further when RO was added to the LF diet. The increased milk protein yield with LF in the current study may be multifaceted. First, this increase may be related to the greater CP concentration in LF diets than the HF diets, which along with the 8% greater DM intake, both contribute to greater protein available for milk protein synthesis. Martineau et al. (2013) reported that milk protein responded positively to canola meal when cows were fed grass forages as in our study cows fed LF consumed a diet with greater rapeseed meal proportion than HF diets. Second, greater milk protein yield with LF compared with HF diets arises mainly from greater milk yield in association with greater starch intake which are expected to promote microbial protein synthesis (Sterk et al., 2011; Roman-Garcia et al., 2016). Specially, feeding diets with more starch compared with NDF as an energy source result in increased net splanchnic release of amino acids, post-hepatic availability of amino acids, and mammary utilization of essential amino acids for milk protein synthesis (Cantalapiedra-Hijar et al., 2014; White et al., 2016). Additionally, the greater energy content may have caused the increased milk protein production (RO supplement in our study) as described by Morris et al. (2020). Not only energy supply, but also the nature of energy supply (glucogenic vs. ketogenic nutrients) can influence milk protein synthesis (Emery, 1978). Milk lactose yield increased for LF compared with HF, and RO supplementation increased it further when included in the LF diet. As milk lactose is the major driver of milk yield due to osmotic pressure, increased lactose synthesis typically increases milk yield (Cant et al., 1993). Furthermore, reduction in de novo synthesis of FA in the mammary gland has the potential to reduce the glucose demand for fat synthesis. If glucose supply is adequate, the “spared” glucose could then be used by other tissues or for lactose synthesis in the mammary gland resulting in increased milk yield (Vojgt et al., 2005) as observed in the current study. It seems that, in our study, increase in efficiency of feed utilization for milk production with RO supplementation to the LF diet could be related to the increase in energy concentration of the diet and the concomitant decline in methane energy losses, which apparently supported milk production.

#### 4.3. Energy and nitrogen utilization

Although our objectives included measurements of energy utilization towards body reserves, BW changes are not reported in the current experiment because changes in BW may not necessarily reflect changes in tissue energy (NRC, 2001) and due to the short experimental periods, the measured BW changes are not reliable. Forage-to-concentrate ratio (65:35 vs. 35:65) and RO supplementation (0 vs. 50 g/kg, on DM basis) did not change the energy balance of the cows. However, we observed a reduced methane energy

excretion as a proportion of energy intake when concentrate was substituted for forage. In agreement with our results, Bayat et al. (2017) reported lower methane energy excretion as a proportion of energy intake from cows fed high-concentrate compared with low-concentrate (304 vs. 126 g/kg starch on a DM basis, respectively). Similarly, Lovett et al. (2003) using heifers reported a significant reduction in methane energy excretion as a proportion of energy intake when concentrate proportion increased in the diet.

Rapeseed oil reduced methane energy excretion as a proportion of energy intake (kJ/MJ) by 3.0% and 2.7% per additional 10 g/kg supplemental oil with HF and LF diets, respectively. Beauchemin and McGinn (2006) found a significant reduction in methane loss as proportion of GE when feeding a diet with 46 g/kg rapeseed oil added to heifers but observed a depression in feed intake. It appears that both forage type and FC ratio are effective on methane energy excretion responses to oil addition to the diet. In lactating cows, Benchaar et al. (2015) reported supplementation of the silage-based diets with 4% DM of linseed oil caused decrease in enteric methane production expressed as a percentage of GE intake, with a more pronounced decrease for cows fed corn silage- than red clover silage-based diets (23 vs. 11%). While increasing the FC ratio also reduced ruminal methane emissions in the reports from lactating cows where forages were replaced by concentrates in the diet (Ferris et al., 1999; Aguerre et al., 2011). Bayat et al. (2017) showed that sunflower oil was more effective in reducing methane emissions when added to low- than high-concentrate diets, whereas in the current study this variable reduced similarly in cows fed HFO and LFO diets. In the current study, the main changing factor in experimental diets is grass silage whereas changes in starch content are marginal for lactating dairy cows (8 vs. 14.5% diet DM). Therefore, changes in grass silage ratios and fermentation quality can affect NDF digestion, rumen fermentation, methane production, and finally energy utilization (Huhtanen et al., 2009). We highlight that the grass silage used in our experiment had relatively high quality as indicated by its chemical composition. This has minimized the difference between silage and supplemental concentrate quality that consequently might have caused minimum differences in energy metabolism when the silage was replaced by concentrates. We hypothesized that dietary oil supplementation would increase the efficiency of ME utilization for milk production because incorporation of dietary fat into milk is more energetically efficient than de novo lipogenesis (Baldwin et al., 1985; Morris et al., 2020). In our study, although the efficiency of converting ME into milk energy was not different between diets, the ratio of ME intake to GE intake was greater for the cows fed LF diets compared with HF diets, which agrees with our previous observations (Bayat et al., 2017). The greater proportion of concentrate in the diets and the inclusion of RO supplementation did not change energy partitioning toward milk synthesis as evaluated by milk energy as a proportion of ME intake, while cows fed LF diets (especially LFO) showed a greater milk yield. The high quality of grass silage and consequently less difference with the supplemental concentrates might have contributed to the lack of effect on energy partitioning.

Nitrogen balance was not different among treatments, whereas previously we observed a positive effect of greater proportion of concentrates or dietary starch on N balance (Aguerre et al., 2011; Morris et al., 2020). In this study, regardless of RO supplementation, cows fed LF diets had lower daily N excretion in feces as a proportion of N intake while they had greater N intake compared with HF diets (688 vs. 558 g/d). This caused improved efficiency of N utilization in LF than HF cows. Even though high proportion of dietary concentrates may stimulate microbial protein synthesis in the rumen and increase intestinal absorption of essential amino acids leading to higher milk protein synthesis, in the current study, FC ratio did not change milk N secretion as a proportion of N intake despite the difference in dietary CP concentration (on average 148 vs. 168 g/kg for HF and LF diets, respectively). However, feeding RO increased efficiency of dietary N utilization, defined as the ratio of milk N output to N intake. Our result is consistent with Hassanat and Benchaar (2021) who demonstrated that supplementing linseed oil (20–40 g/kg DM) led to improved efficiency of dietary N utilization due to simultaneous decreases in N intake and increases in milk N secretion as proportion of N intake. Based on these observations, the increase in efficiency of N utilization may also be explained by the decrease in dietary CP concentration when RO was added to the diets. Further, the partitioning of N towards milk in cows fed RO-supplemented diets was likely related to an increase in dietary energy content which is consistent with the observations of Rius et al. (2010).

#### 4.4. Feed and milk fatty acid compositions

Dietary addition of RO increased the intakes of the most dietary FA (including 18:0, *cis*-9 18:1, *cis*-9, *cis*-12 18:2, and *cis*-9, *cis*-12, *cis*-15 18:3) with a different magnitude when used in LF and HF diets. These variations were reflected in milk fat where concentrations of the most of these FA were increased when cows received RO. Similarly, feeding LF diets increased the intake of *cis*-9, *cis*-12 18:2 which explains the higher concentration of this essential FA in milk fat as compared with HF diets.

The main factor in the variation of BH in the rumen is the FC ratio of the diet, and basal diet appears to have a profound effect on ruminal metabolism of FA when diet is supplemented with oil sources (Shingfield et al., 2005). Feeding a low FC ratio diet markedly affects the ratio of cellulolytic to propionigenic, lactogenic, and amylolytic bacteria, which in turn affects ruminal BH (Chilliard et al., 2007). Consequently, interactions between level of oil supplementation and other dietary changes are likely to occur. In the present study, the concentration of SFA in milk fat was negatively affected by RO supplementation but a significant interaction effect with FC ratio was observed; with more pronounced decrease in SFA from cows fed LFO than HFO diet. In particular, this trend was observed for 8:0, 10:0, and 12:0 which are the SFA in milk, and, to a minor extent, for 4:0 and 14:0. Previous research reported similar effects of dietary RO on milk FA composition using similar amount of RO in the diet with 60:40 FC ratio, and the effects were of similar magnitude (Bayat et al., 2018). Stearic acid (18:0) increased in the milk fat by feeding RO due to the abundance of 18-carbon UFA in RO diet which is hydrogenated to 18:0 in the rumen. Likewise, Kliem et al. (2019) reported a reduction in milk fat SFA concentration with 59 g/kg DM of milled rapeseed (500 g/d oil) added to a maize silage/grass silage-based diet with 50:50 FC ratio, but the reduction was mainly due to a significant reduction of 16:0 in milk fat. In a previous experiment (Leskinen et al., 2019), sunflower oil supplementation (50 g/kg DM) tended to decrease SFA in milk fat more when supplemented in low-forage diet (35:65) compared with high-forage diet (65:35), which is consistent with the present experiment, although the magnitude of the changes were different. Milk

SFA were mainly replaced with *cis*-MUFA following oil supplementation, the most predominant being *cis*-9 18:1. Intake of *cis*-9 18:1 was high for the HFO and LFO, and the appearance of *cis*-9 18:1 in milk is associated with both increased intake and increased rumen outflow of 18:0 that is subsequently desaturated to *cis*-9 18:1 by mammary  $\Delta$ -9 desaturase (Bauman and Griinari, 2003). Kliem et al. (2019) reported a smaller increase in *cis*-9 18:1 in milk fat than in the current study, but the amount of oil from rapeseed in the diet was also lower, whereas in the study of Bayat et al. (2018) the increase in milk *cis*-9 18:1 was greater with similar amount of RO in the diet.

The effect of FC ratio on milk 18:1 FA composition differed, depending on the presence or absence of RO in the basal diet which was expected. Among the intermediates of ruminal BH, the level of *trans*-11 18:1 increased more by feeding HFO compared with LFO diet, but an inverse interaction was observed on the concentration of *trans*-10 18:1. Feeding lower proportion of forage promotes the alternate (*trans*-10) BH pathway instead of the normal pathway (*trans*-11; Shingfield et al., 2010) as reported in the current trial as well as in previous experiments (Sterk et al., 2011; Saliba et al., 2014). In many cases, reductions in milk fat secretion have consistently been associated with increases in milk *trans*-10, *cis*-12 CLA and, in some cases, in milk *trans*-10 18:1 (Shingfield et al., 2010). In the present experiment, an interaction effect between FC ratio and RO addition was observed on milk fat concentration but not on milk fat yield. Compared with other diets, the milk fat concentration reduced in cows fed LFO by 19% which may be explained by the inhibitory effect of *trans* FA biohydrogenation intermediates on milk fat synthesis. The concentrations of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA in milk fat increased by 89% and 80% of total FA, respectively, in cows fed LFO compared with other diets. The greater concentration of alternate BH intermediates (i.e., 3.25% *trans*-10 18:1) in milk from cows fed LFO originated from enhanced diet fermentability and PUFA content.

Feeding RO increased *cis*-9, *trans*-11 CLA concentration in milk fat. The *cis*-9, *trans*-11 CLA is produced mainly endogenously by the mammary desaturation of *trans*-11 18:1 by stearoyl-CoA desaturase enzyme but also as an intermediate in rumen BH (Bauman and Griinari, 2003; Chilliard et al., 2007). The concentration of *cis*-9, *trans*-11 CLA, in fact, more than doubled in milk from cows fed HFO and LFO diets compared with HF and LF diets. However, the level of CLA enrichment was greater in the present study than in previous trials (Altenhofer et al., 2014; Kliem et al., 2019) where dairy cow diets were supplemented with vegetable oil. This result might be attributed to the greater amount of RO supplemented in the present trial.

## 5. Conclusions

Low-forage diet and rapeseed oil supplement fed together increased the yields of milk, milk protein and lactose compared with other diets, while it decreased milk fat concentration. Energy intake lost as methane was lower whereas ME intake was greater for the low- compared with high-forage diets. Rapeseed oil decreased the excretion of methane energy as a proportion of energy intake in both low- and high-forage diets which was explained, at least in part, by decreases in feed intake. Further, dietary rapeseed oil supplementation increased the partitioning of N towards milk protein synthesis on low-forage diets in this study. Diets did not influence energy and N balances. The low-forage diet combined with rapeseed oil supplement increased milk *trans*-10 18:1 and *trans*-10, *cis*-12 18:2, resulting in the lowest milk fat concentration without influencing milk fat yield compared with other diets. Rapeseed oil increased *cis* monounsaturated FA, while feeding low-forage diet with rapeseed oil decreased saturated FA and increased total *trans* FA in milk fat. Overall, higher dietary concentrate ratio was effective in improving energy partitioning towards milk, whereas rapeseed oil supplement improved N partitioning towards milk when supplemented to both high- and low-forage diets.

## CRedit authorship contribution statement

**Ali Razzaghi:** Formal analysis, Writing – original draft, Writing – review & editing. **Heidi Leskinen:** Writing – review & editing. **Seppo Ahvenjärvi:** Writing – review & editing. **Heikki Aro:** Writing – review & editing. **Ali Reza Bayat:** Conceptualization, Methodology, Supervision, Writing – review & editing.

## Conflicts of interest

The authors confirm that there are no recognized conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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