Dietary forage to concentrate ratio and sunflower oil supplement alter rumen fermentation, ruminal methane emissions, and nutrient utilization in lactating cows[1]

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ABSTRACT: The effects of supplementing high- or low-concentrate diets with sunflower oil (SO) on rumen fermentation, nutrient utilization, and ruminal methane (CH_4) emissions in lactating cows were examined. Four multiparous Nordic Red dairy cows fitted with rumen cannulae were used in a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments and 35-d periods. Experimental treatments comprised iso-nitrogenous total mixed rations based on grass silage with forage to concentrate ratio of 65:35 or 35:65 supplemented with 0 or 50 g/kg diet DM of SO. Apparent ruminal OM and starch digestibility was greater ($P < 0.05$) with high- than low-concentrate diets but was unaffected by SO. Inclusion of SO in high-concentrate diet decreased ($P \leq 0.05$) apparent total tract OM, fiber, and GE, and apparent ruminal fiber digestibility. High-concentrate diets and SO shifted ($P < 0.05$) fiber digestion from rumen to the hindgut. High-concentrate diet resulted in a lower rumen pH and elevated total rumen VFA concentration compared with low-concentrate diet, whereas SO increased rumen pH and decreased rumen VFA concentration when included in high-, but not low-concentrate diet ($P < 0.05$ for interaction). High-concentrate diet reduced rumen ammonia-N ($P < 0.01$) and molar proportion of acetate to propionate ($P < 0.01$), and decreased ($P < 0.05$) ruminal CH_4 emissions when expressed as g/d or g/kg OM digested in the rumen. With both low- and high-concentrate diets, SO reduced ($P < 0.05$) daily emissions of CH_4 as g/d or g/kg OM digested in the rumen, but SO reduced CH_4 emissions expressed as g/kg OM intake, OM digested in total digestive tract, energy-corrected milk or % of GE intake only with low-concentrate diet ($P \leq 0.05$ for interaction). In conclusion, replacing grass silage with concentrates led to a reduction in daily ruminal CH_4 emissions that were accompanied by a shift in rumen fermentation toward the synthesis of propionate, and decreases in rumen pH and fiber digestion. Sunflower oil was effective in reducing daily CH_4 emissions in lactating cows which was accompanied by a noticeable lower feed intake with high- but not low-concentrate diet. Overall the effects of SO and greater proportion of concentrates in the diet on daily CH_4 emissions were additive but the additivity declined or vanished when different indices of CH_4 emission intensity were considered. Consequently, SO was more effective in reducing CH_4 emissions when low-concentrate diet was fed.

Key words: dairy cow, digestibility, forage to concentrate ratio, ruminal methane, sunflower oil

Transl. Anim. Sci. 2017.1:277–286
doi:10.2527/tas2017.0032

[1]The authors express their appreciation to Natural Resources Institute Finland (Luke; formerly MTT Agrifood Research Finland) staff in the metabolism unit for technical support, care of experimental animals, and assistance in sample collection coordinated by Mari Talvisilta and in the laboratory for the chemical analysis of samples under the supervision of Taina Jalava. Authors gratefully acknowledge funding provided by the Finnish Ministry of Agriculture and Forestry (MMM; Helsinki) as part of the GreenDairy Project (Developing Genetic and Nutritional Tools to Mitigate the Environmental Impact of Milk Production; Project No. 2908234).

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[3]Deceased. The authors dedicate this article to the memory of Kevin Shingfield.

Received January 13, 2017.
Accepted May 10, 2017.

INTRODUCTION

Global demand for food will increase over the next decades (Opio et al., 2013). Even though ruminants are capable of converting non-edible organic material into high quality human food, their notable contribution to greenhouse gas (GHG) emissions and excretion of nitrogen into the environment remains challenging. Consequently, there is a considerable interest in reducing GHG emissions from ruminant production systems. Enteric methane (CH_4) production is the major source of GHG emissions from ruminants that represents a loss of 2 to 12% of GE intake (Johnson and Johnson, 1995). Several nutritional strategies have shown potential for reducing enteric CH_4 production that include replacing dietary fiber with starch, using forages of greater digestibility and dietary supplements of oils, condensed tannins, saponins, sulfate, or nitrate (Martin et al., 2010; Knapp et al., 2014). The impact of oils and oilseeds rich in unsaturated fatty acids on rumen fermentation and digestion are known to be dependent on the composition of the basal diet (Jenkins, 1993). This implies that the efficacy of plant oils rich in PUFA in reducing enteric CH_4 production in ruminants might be dependent on the relative proportion of forage and concentrates in the basal diet. Identifying dietary strategies that have additive CH_4 mitigating effects would form a sustainable strategy to reduce the carbon footprint of the dairy production systems. Whether combination of forage to concentrate (FC) ratio or plant oils in the diet has additive effect on ruminal CH_4 emissions is so far unclear. We hypothesized that lower dietary FC ratio and dietary supplement of sunflower oil (SO) have additive effects on ruminal CH_4 emissions. The objectives were to evaluate the effects of dietary FC ratio and supplement of SO on rumen fermentation, nutrient digestibility and utilization, and ruminal CH_4 emissions.

MATERIALS AND METHODS

All experimental procedures were approved by the National Ethics Committee ESAVI/794/04.10.03/2011, Kuopio, Finland) in accordance with the guidelines established by the European Community Council Directive 86/609/EEC (European Union, 1986).

Animals, Experimental Design, and Diets

Details on experimental animals, treatments, and experimental design have been reported (Ventto et al., 2017). In brief, 4 multiparous Nordic Red cows in mid-lactation (89 ± 11.8 d in milk) producing 32.1 ± 1.3 kg milk/d and fitted with rumen cannulae (#1C, i.d. 100 mm; Bar Diamond Inc., Parma, ID) were used in a 4 × 4 Latin square with 2 × 2 factorial arrangement of treatments. Each experimental period comprised 14-d diet adaptation, 12-d sampling interval, and 9-d washout. Treatments consisted of iso-nitrogenous total mixed rations (TMR) based on grass silage containing a low (65:35 FC) or high (35:65 FC) proportion of concentrates supplemented with 0 (L and H, respectively) or 50 g/kg diet DM of SO (LSO and HSO, respectively). Sunflower oil (Tuko Logistics Ltd., Kerava, Finland) was stored in 4°C until incorporated into the low- or high-concentrate TMR and the oil replaced concentrate ingredients. Formulation of dietary concentrates and the chemical composition of grass silage and concentrates are presented in Table 1. Experimental silage was prepared from primary growths of timothy (*Phleum pratense)* and meadow fescue (*Festuca pratensis*) and ensiled with a formic acid-based additive. Grass silage contained (g/kg as fed) 246 DM, and (g/kg DM) OM 930, CP 136, NDF 508, indigestible NDF (iNDF) 103, water soluble carbohydrates (WSC) 27.0, starch 4.1, and GE of 18.6 MJ/kg DM. Diets were offered 4 times daily at 0600, 0900, 1630, and 1930 h and fed as a TMR to avoid selection of dietary components and maintain the desired FC ratio. Experimental diets were offered ad libitum to result in 10% refusals and formulated to meet or exceed ME and MP requirements of lactating cows producing 30 kg milk/d (MTT Agrifood Research Finland, 2006). Cows were housed in individual tie stalls in a dedicated metabolism unit with free access to water and salt block.

Feed Intake and Milk Production

Daily feed intake and milk yield were measured during d 22 to 26 of each experimental period. During this period, representative samples of silage and concentrates were collected daily, composited, and submitted for determination of chemical composition as described by Shingfield et al. (2002). Silage DM content was corrected for the loss of volatiles according to Huida et al. (1986). For each experimental TMR, chemical composition was calculated based on the analysis of silage, concentrate, and SO components. The GE of silage, concentrates, SO, and excreta was determined by bomb calorimetry (1108 Oxygen bomb, Parr Instrument Co., Moline, IL). Concentration of iNDF in silage, concentrates, and feces was determined in duplicate by incubation of 1.0 g of sample DM in nylon bags (60 × 120 mm, pore size 0.017 mm) within the rumen of 2 cows fed a grass silage-based diet (FC ratio 70:30 on a DM basis) for 12 d. Once removed from the rumen, bags were rinsed in cold water for 25 min using a household washing machine, incubated for 1 h in boiling neutral detergent solution, rinsed, and dried to a constant weight at 60°C. Potentially digestible NDF (pdNDF) was calculated as NDF- iNDF.

Table 1. Formulation and chemical composition of experimental diets[1]

	Diet[2]			
Item, unit	L	LSO	H	HSO
Inclusion, g/kg DM				
Grass silage[3]	650	650	350	350
Rolled barley	55	42	130	116
Ground wheat	165	126	390	352
Rapeseed expeller[4]	100	100	100	100
Urea[5]	0	2	0	2
Sunflower oil[6]	0	50	0	50
Vitamin and mineral premix[7]	30	30	30	30
Chemical composition, g/kg DM[8]				
DM, g/kg as fed	474	479	668	672
OM	914	915	928	929
CP	154	153	150	150
NDF	386	378	267	262
iNDF	82.2	80.4	59.0	58.0
WSC	32.6	31.0	31.6	31.2
Starch	143	110	318	290
GE, MJ/kg DM	18.6	19.7	18.8	20.1
Fatty acid composition, g/100 g fatty acid				
12:0	0.11	0.03	0.07	0.02
14:0	0.36	0.15	0.31	0.14
16:0	14.6	8.27	14.8	8.4
16:1	1.21	0.41	0.80	0.31
18:0	1.50	3.28	1.40	3.22
18:1	22.9	27.1	24.3	27.7
18:2n-6	28.3	50.8	38.2	53.1
18:3n-3	25.5	7.07	16.2	4.7
SFA	19.9	13.6	18.8	13.4
MUFA	25.9	28.1	26.4	28.5
PUFA	54.2	58.3	54.8	58.1
Total fatty acids, g/kg DM	17.7	64.6	18.5	65.6

[1]Reprinted from Ventto et al. (2017), The Nutrition Society, published by Cambridge University Press.

[2]Refers to the concentrate designated to the diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LSO and HSO, respectively) g/kg DM of sunflower oil.

[3]Restrictively fermented grass silage prepared from the primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards (54:46, respectively), grown at Jokioinen (60°49′ N, 23°28′E) treated with a formic acid based ensiling additive (0.76 formic acid and 0.055 ammonium formate, AIV 2 Plus; Valio Ltd., Helsinki, Finland). Mean fermentation characteristics pH 3.95; in DM (g/kg); lactic acid 63.5, acetic acid 22.1, propionic acid 0.18, formic acid 19.1, and water-soluble carbohydrate 27.0, soluble N (g/kg total N) 634 and ammonium N (g/kg total N) 63.9. Grass silage contained 228 g/kg DM (as fed).

[4]Prepared from rapeseeds of low glucosinolate concentrations (Avena Nordic Grain Ltd., Espoo, Finland).

[5]Urea containing (g/kg DM) OM (1,000) and CP (2,899), 15604 Urea, Sigma-Aldrich, Stockholm, Sweden.

[6]Sunflower oil containing (g/100 total fatty acids) 16:0 (6.14), 18:0 (3.91), *cis*-9 18:1 (27.9), *cis*-11 18:1 (0.66), and 18:2n-6 (59.1) as major components (Tuko Logistics Ltd., Kerava, Finland).

[7]Declared as containing (g/kg) calcium (190), magnesium (60), sodium (135), zinc (2.19), manganese (0.45), copper (0.40); (mg/kg), iodine (55), cobalt (35), selenium (30), and dl-tocopheryl acetate (550); (IU/kg) retinyl acetate (220,000), and cholecalciferol (40,000), Onni, Melica Finland Ltd., Vaasa, Finland.

[8]iNDF, indigestible NDF; WSC, water soluble carbohydrates.

Cows were milked twice daily at 0700 and 1645 h. Milk samples were collected over 8 consecutive milking starting at 1645 h on d 24, treated with preservative (Bronopol, Valio Ltd., Helsinki, Finland) and milk fat, CP, and lactose were predicted by infrared analysis (MilkoScan 133B, Foss Electric, Hillerød, Denmark). Milk composition was calculated based on the weighted average of morning and afternoon milk yields.

Rumen Fermentation

Samples of ruminal fluid (150 mL; $n = 8$) were collected using a suction pump equipped with a Büchner flask through the rumen cannulae on d 26 of each period at 1.5 h intervals from 0600 until 1630 h. Following the collection of rumen liquid, pH was measured and samples were filtered through 2 layers of cheesecloth. For VFA determinations, 5.0 mL ruminal fluid was preserved with 0.5 mL of saturated $HgCl_2$ and 2.0 mL of 1 M NaOH. Additional samples of ruminal fluid (15.0 mL) were also collected, preserved with 0.3 mL of 50% (vol/vol) sulfuric acid and submitted for the analysis of ammonia-N concentrations. Samples were stored at –20°C until analyzed for VFA and ammonia-N determinations as described by Shingfield et al. (2002).

Apparent Digestibility and Nutrient Utilization

Nutrient flow at omasum was measured using the omasal sampling technique and Cr, Yb, and iNDF as indigestible markers over 3 d 4 times daily at 3-h intervals with advancing 1 h each day based on the methods described by Ventto et al. (2017). Omasal nutrient flow was used to calculate the apparent ruminal digestibility of nutrients. Total tract apparent digestibility coefficients were determined by feces collected over a 96-h interval starting at 1800 h on d 21 of each experimental period. Representative samples were collected daily and composited for every cow within each period. The samples were dried in oven (55°C, 48 h) and submitted for determination of chemical composition as for the feed samples. Urine was separated from feces by means of a light harness and flexible tubing attached to the vulva and collected in plastic canisters containing 500 mL of 5 mol/L sulfuric acid. Urine was subsampled, composited across sampling days, and freeze-dried (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) prior to GE determination by bomb calorimetry and N determination by Kjeldahl method.

Ruminal Gas Production

Ruminal CH_4 and carbon dioxide (CO_2) emissions were measured over 6 d (d 16 to 22 of each pe-

riod) using the sulfur hexafluoride (SF_6) tracer technique (Boadi et al., 2002) with minor modifications described earlier by Bayat et al. (2015). In brief, gases in the rumen headspace were drawn continuously (1.7 mL/min) over every 24-h period into evacuated 5.5 L air-tight canisters through a capillary tubing (PEEK 1.6 mm × 0.13 mm i.d., VICI Valcro Instruments Co, Houston, TX). Tubes used to collect the ruminal gas were anchored securely to the neck of the rumen cannula allowing gas to be collected at approximately 5 cm above the rumen mat. Subsamples of ruminal gases were analyzed in triplicate for CH_4, CO_2, and SF_6 concentrations by gas chromatography (Agilent 6890N, Agilent Technologies, Santa Clara, CA) as described by Regina and Alakukku (2010). Daily ruminal CH_4 and CO_2 emissions were calculated based on the measured SF_6 release rate (1.11 ± 0.20 mg/d) in the rumen over the course of experiment and concentrations of CH_4, CO_2, and SF_6 in analyzed rumen gases.

Calculations

Ruminal or total tract digestibility coefficients were calculated based on the difference between intake and flow at the omasum or output in feces, respectively. Intake of ME was calculated as the difference between GE intake and energy excretion in feces, urine, and CH_4. Energy losses as CH_4 were calculated using the factor 55.24 kJ/g (Kriss, 1930). Energy secretion in milk was calculated based on the yields of fat, CP, and lactose (Sjaunja et al., 1990). Nitrogen balance was calculated as the difference between N intake and N excretion in feces, urine, and milk where milk N was calculated as milk CP/6.38.

Statistical Analysis

Experimental data were analyzed by ANOVA for a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments using the Mixed procedure of SAS (version 9.2, SAS Inst. Inc., Cary, NC) with a model that included the fixed effects of period, FC, SO, and FC by SO interaction, and random effect of cow. Least square means ± SEM are reported. Effects of FC, SO, and their interaction were declared significant at $P \leq 0.05$. Probabilities at $0.05 < P < 0.10$ were considered as a trend.

RESULTS

Nutrient Digestion and Metabolism

Apparent ruminal OM digestibility was unaffected ($P = 0.14$) by SO supplement, but it was greater ($P < 0.01$) with H than L diets (Table 2). Dietary supplement of SO tended to reduce ($P < 0.07$ for FC × SO interaction) apparent ruminal NDF and pdNDF digestion and decreased ($P \leq 0.05$ for FC × SO interaction) total tract OM, NDF, pdNDF, and GE digestibility when included in H but not L diet. Inclusion of SO decreased ($P < 0.05$) ruminal N digestibility, whereas treatments had no effect ($P = 0.26$) on total tract N digestibility. Ruminal ($P < 0.05$) and total tract ($P = 0.06$) starch digestion were greater when H than L diets were fed. Both greater dietary proportion of concentrates ($P < 0.05$) and inclusion of SO supplement ($P \leq 0.062$) shifted fiber digestion from the rumen to the hindgut as indicated by the lower proportion of ruminal digestibility.

The data on nutrient intake, milk yield and milk composition used for further calculations in this paper were reported and discussed earlier by Ventto et al. (2017) and are presented in Table 2 and Table 3, respectively. Intake of GE was greater ($P < 0.01$) when H compared with L diets were fed, but was not altered ($P = 0.78$) by SO supplement (Table 3). The proportion of GE intake excreted as urine was greater ($P < 0.01$) when L compared with H diet was fed, whereas SO supplement had no effect ($P = 0.10$). The proportion of GE intake lost as CH_4 was greater ($P < 0.05$ for FC × SO interaction) for L than H diet, and SO reduced this proportion more profoundly when supplemented to L compared with H diet. Milk energy as a proportion of ME intake was reduced ($P < 0.05$) in response to both greater proportion of concentrates in the diet and SO supplement.

Intake of N was greater ($P < 0.01$) when H compared with L diets were fed and tended ($P = 0.09$) to be decreased by SO supplement (Table 3). Urinary N excretion as a proportion of N intake was smaller ($P < 0.05$) when H compared with L diets were fed. Dietary supplement of SO decreased the ratio of milk N/N intake when included in L diet, whereas the reverse was true with H diet ($P < 0.01$ for FC × SO interaction). Nitrogen balance was improved ($P < 0.01$) by the greater proportion of concentrates but inclusion of SO tended ($P = 0.065$ for for FC × SO interaction) to improve the N balance when included in L diet while the reverse was true with H diet.

Rumen Fermentation

Greater proportion of concentrates in the diet resulted in a lower rumen pH and elevated rumen VFA concentrations, whereas SO increased rumen pH and decreased rumen VFA concentrations when included in H, but not L diet ($P < 0.05$ for FC × SO interaction; Table 4). The cows fed H diets had lower ($P < 0.01$) rumen ammonia-N concentration compared with L diets. Molar proportions of acetate and isobutyrate were lower ($P < 0.05$) and those of propionate and valerate

Table 2. Effects of forage to concentrate ratio and dietary sunflower oil supplement on intake, and apparent ruminal and total tract nutrient digestibility in lactating dairy cows

	Treatment[1]					*P*-value[3]		
Item, unit	L	LSO	H	HSO	SEM[2]	FC	SO	FC×SO
Intake, kg/d[4]								
DM	19.0	18.6	23.3	20.7	0.78	0.004	0.088	0.18
OM	17.3	16.9	21.6	19.2	0.72	0.003	0.090	0.18
CP	2.90	2.82	3.49	3.10	0.122	0.009	0.088	0.22
NDF	7.95	7.66	6.73	5.89	0.257	0.001	0.050	0.29
pdNDF[5]	6.37	6.14	5.32	4.66	0.201	0.001	0.051	0.30
WSC[6]	0.32	0.28	0.53	0.46	0.018	0.010	0.040	0.37
Starch	2.57	1.94	7.18	5.83	0.167	0.001	0.001	0.042
Apparent ruminal digestibility, %								
OM	39.6	37.0	43.4	42.3	1.54	0.005	0.14	0.59
CP	–13.8	–10.7	–16.4	–6.6	2.77	0.78	0.049	0.24
NDF	50.6	48.7	39.3	30.2	1.64	< 0.001	0.016	0.069
pdNDF	64.2	61.7	50.3	38.7	1.98	< 0.001	0.012	0.059
Starch	85.6	80.3	90.9	89.5	3.63	0.038	0.24	0.47
Apparent total tract digestibility, %								
OM	70.9	71.0	75.7	72.2	0.384	< 0.001	0.003	0.002
CP	67.5	69.2	68.9	69.7	1.00	0.38	0.26	0.72
NDF	57.8	59.3	50.9	44.5	1.43	< 0.001	0.12	0.026
pdNDF	70.2	69.6	57.2	48.6	1.53	< 0.001	0.009	0.016
Starch	98.8	98.4	99.2	98.9	0.21	0.059	0.17	0.91
GE	66.9	68.3	73.2	69.9	1.10	0.007	0.36	0.053
Ruminal proportional digestibility, %								
OM	55.9	52.6	57.4	58.6	2.16	0.028	0.44	0.13
NDF	87.6	82.3	76.9	67.2	2.45	0.002	0.022	0.40
pdNDF	91.4	88.6	87.7	78.8	2.56	0.039	0.062	0.28
Starch	86.6	81.6	92.0	90.5	2.45	0.041	0.25	0.51

[1]Refers to diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LSO and HSO, respectively) g/kg of sunflower oil on a DM basis.

[2]n = 16 measurements; error degrees of freedom 6.

[3]FC, effect of forage to concentrate ratio in the diet; SO, effect of sunflower oil supplement; FC × SO, interaction of FC and SO.

[4]Intake data is reprinted from Ventto et al. (2017), The Nutrition Society, published by Cambridge University Press.

[5]pdNDF, potentially digestible NDF.

[6]WSC, water soluble carbohydrate.

were higher ($P < 0.05$) with H compared with L diets. The molar acetate / propionate ($P < 0.01$) or acetate + butyrate / propionate ($P < 0.05$) ratios were lower with H compared with L diets but were not affected ($P \geq 0.84$) by SO supplementation.

Ruminal Gas Emissions

The greater proportion of concentrates and inclusion of SO in the diet reduced ($P < 0.05$) daily ruminal CH_4 emissions by 13.5 and 22.2% (Table 5) corresponding to decreases in CH_4 per kg OM apparently digested in the rumen of 34.3% ($P < 0.01$) and 14.8% ($P = 0.06$), respectively. The CH_4 emissions intensity calculated as g/kg OM intake, OM digested in total digestive tract, ECM, or as a proportion of GE intake was greater for L than H, and inclusion of SO in L diet was more potent in reducing CH_4 emissions intensity than H diet ($P \leq 0.05$ for FC × SO interaction). Supplement of SO tended ($P = 0.07$ for FC × SO interaction) to result in greater decreases in daily ruminal CO_2 emissions when included in L than H diet. Inclusion of SO in L diet decreased ($P < 0.05$ for FC × SO interaction) CO_2 per kg OM intake, per kg OM digested in the rumen or total digestive tract, and per kg ECM, parameters that were all increased when SO was included in H diet.

DISCUSSION

Nutrient Digestion and Metabolism

The greater proportion of concentrates in the diet was associated with more extensive digestion of OM in the rumen that reflects the greater intrinsic digestibility of non-structural carbohydrates compared with

Table 3. Effects of forage to concentrate ratio and dietary sunflower oil supplement on milk yield, and energy and nitrogen partitioning in lactating dairy cows

	Treatment[1]					*P*-value[3]		
Item, unit	L	LSO	H	HSO	SEM[2]	FC	SO	FC×SO
Milk yield, kg/d[4]	26.7	25.7	29.7	28.9	2.50	0.12	0.60	0.97
Energy corrected milk, kg/d	26.1	25.5	30.0	25.1	1.99	0.18	0.056	0.10
Fat, g/d	1,050	1,076	1,230	838	84.6	0.55	0.007	0.003
Protein, g/d	901	823	1013	1012	60.9	0.005	0.42	0.38
Lactose, g/d	1161	1122	1292	1257	115	0.14	0.64	1.00
Energy								
GE intake, MJ/d	353	365	438	417	16.0	0.003	0.78	0.30
Energy output/energy intake, %								
Feces	33.1	31.7	26.8	30.1	1.10	0.007	0.36	0.053
Urine	4.14	3.77	3.10	2.80	0.175	0.001	0.10	0.85
Methane	7.20	5.12	4.90	4.36	0.304	0.002	0.005	0.044
Milk	23.3	22.1	21.5	18.7	1.59	0.010	0.030	0.31
ME intake/GE intake	55.0	59.0	65.0	62.6	1.26	0.001	0.54	0.034
Milk energy/ME intake	42.3	37.4	33.0	29.8	2.47	0.002	0.037	0.59
Nitrogen (N)								
N intake, g/d	464	452	558	496	19.6	0.009	0.09	0.22
N output/N intake, %								
Feces	32.5	30.8	31.1	30.3	1.00	0.38	0.26	0.72
Urine	36.9	37.1	30.0	31.0	2.60	0.022	0.78	0.86
Milk	30.5	28.7	29.3	33.0	1.60	0.066	0.19	0.006
N balance, g/d	1.50	16.1	53.6	29.3	8.62	0.009	0.59	0.065

[1]Refers to diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0.0 (L and H, respectively) or 50 (LSO and HSO, respectively) g/kg of sunflower oil on a DM basis.

[2]n = 16 measurements; error degrees of freedom 6.

[3]FC, effect of forage to concentrate ratio in the diet; SO, effect of sunflower oil supplement; FC × SO, interaction of FC and SO.

[4]Milk yield data is reprinted from Ventto et al. (2017), The Nutrition Society, published by Cambridge University Press.

Table 4. Effects of forage to concentrate ratio and dietary sunflower oil supplement on rumen fermentation characteristics in lactating dairy cows

	Treatment[1]					*P*-value[3]		
Item, unit	L	LSO	H	HSO	SEM[2]	FC	SO	FC×SO
pH	6.61	6.51	6.03	6.26	0.089	< 0.001	0.37	0.033
Ammonia-N, mmol/L	4.90	6.29	2.80	2.70	0.622	0.004	0.34	0.28
Total VFA, mmol/L	109	105	123	109	3.8	0.003	0.004	0.038
Molar proportions, mmol/mol								
Acetate	658	648	594	592	6.7	< 0.001	0.25	0.43
Propionate	180	185	231	224	16.2	0.032	0.96	0.71
Butyrate	111	121	125	130	14.1	0.44	0.64	0.88
Isobutyrate	8.89	8.46	7.20	8.00	0.360	0.021	0.61	0.12
Valerate	16.3	15.2	19.0	19.5	0.67	0.002	0.65	0.28
Isovalerate	15.9	15.2	16.0	19.4	1.86	0.14	0.34	0.16
Caproate	9.41	7.88	7.34	7.28	1.246	0.25	0.47	0.51
Acetate/Propionate	3.69	3.51	2.65	2.74	0.211	0.005	0.84	0.55
Acetate+Butyrate/Propionate	4.31	4.16	3.21	3.37	0.308	0.022	0.98	0.63

[1]Refers to diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LSO and HSO, respectively) g/kg of sunflower oil on a DM basis.

[2]n = 16 measurements; error degrees of freedom 6.

[3]FC, effect of forage to concentrate ratio in the diet; SO, effect of sunflower oil supplement; FC × SO, interaction of FC and SO.

Table 5. Effects of forage to concentrate ratio and dietary sunflower oil supplement on ruminal methane (CH_4) and carbon dioxide (CO_2) emissions in lactating dairy cows

	Treatment[1]					*P*-value[3]		
Item, unit	L	LSO	H	HSO	SEM[2]	FC	SO	FC×SO
Ruminal CH_4								
g/d	492	362	404	335	20.4	0.024	0.002	0.16
g/kg OMI[4]	28.4	21.3	18.7	17.7	1.27	0.002	0.015	0.045
g/kg ruminal DOM[5]	72.5	57.6	43.9	41.6	3.84	0.001	0.06	0.15
g/kg total tract DOM	40.0	30.0	24.7	24.5	1.74	< 0.001	0.021	0.024
g/kg ECM[6]	18.9	14.5	14.2	14.5	1.38	0.051	0.08	0.052
% of GE intake	7.20	5.12	4.90	4.36	0.304	0.002	0.005	0.044
Ruminal CO_2								
g/d	4056	3045	3880	3671	166.9	0.25	0.018	0.07
g/kg OMI	234	177	180	192	9.2	0.09	0.07	0.016
g/kg ruminal DOM	598	465	415	455	29.6	0.027	0.20	0.039
g/kg total tract DOM	331	249	237	266	13.6	0.045	0.13	0.012
g/kg ECM	156	117	136	160	15.3	0.33	0.51	0.036

[1]Refers to diets based on low (0.35) or high (0.65) concentrate ratio supplemented with 0 (L and H, respectively) or 50 (LSO and HSO, respectively) g/kg of sunflower oil on a DM basis.

[2]n = 16 measurements; error degrees of freedom 6.

[3]FC, effect of forage to concentrate ratio in the diet; SO, effect of sunflower oil supplement; FC × SO, interaction of FC and SO.

[4]OMI, OM intake.

[5]DOM, apparent OM digestion.

[6]ECM, energy-corrected milk.

hemicellulose and cellulose. Supplement of SO had adverse effects on ruminal and total tract fiber digestion and shifted digestion to the hindgut when included in H diet, but not when included in L diet. Earlier experiments have demonstrated that supplements of sunflower or linseed oil often shift digestion of OM to the hindgut (Ueda et al., 2003; Shingfield et al., 2008).

A tendency for greater proportion of starch digestion in the rumen when feeding concentrate-rich diets may be indicative of more favorable conditions for starch-fermenting bacterial species, given that starch-fermenting bacteria are more tolerant to low rumen pH when high-concentrate diets are fed (Goad et al., 1998; Petri et al., 2013). Moreover, part of the difference in ruminal starch digestion can arise from the lower proportional microbial starch flow (endogenous starch) when diet has greater starch content (Huhtanen et al., 2010). A lack of change in starch digestibility in response to SO is consistent with the previous studies that looked into the effects of plant oils on nutrient digestion (Ueda et al., 2003; Shingfield et al., 2011). Both the greater proportion of concentrates in the diet and inclusion of SO supplement reduced energy partitioning toward milk synthesis as evaluated by milk energy as a proportion of ME, without altering milk yield. This suggests a greater proportion of ME was retained in body tissues which is consistent with the observed improvement in N balance.

Rumen Fermentation

Greater proportion of concentrates in the diet reduced rumen pH, which can be explained by more extensive fermentation of OM, and by inference more VFA production. Sunflower oil increased rumen pH when included in H but not L diet, which can be attributed to the decrease in DM intake (Huhtanen and Kukkonen, 1995). Even though experimental diets were iso-nitrogenous and had the same level of rapeseed expeller as protein supplement, rumen ammonia-N concentration was less in animals fed H diets. The main reason for this difference could be the lower proportion of silage, having 634 g soluble N and 63.9 g ammonia-N per kg total N, in H diet. In addition, replacing fibrous carbohydrates in L diets with starch in H diets would be expected to facilitate better utilization of ammonia and reduce the deamination of amino acids by rumen microorganisms (Nocek and Russell, 1988). For rations based on grass silage, increases in dietary soluble sugar or starch content (Chamberlain and Choung, 1995) typically reduce rumen ammonia-N concentration.

The greater proportion of concentrates in the diet reduced the molar acetate and butyrate to propionate ratio in rumen VFA, consistent with some (Jaakkola and Huhtanen, 1993; Moss et al., 1995), but not all studies (Moorby et al., 2006; Owens et al., 2008). Present findings indicate that when fed in relatively

high amounts, concentrate supplements influence molar VFA proportions in lactating cows fed diets based on restrictively fermented grass silage.

Ruminal Gas Emissions

The effects of dietary FC ratio and supplement of SO on daily ruminal CH_4 emissions but not on CH_4 emissions intensity (expressed per unit of intake, OM digested or milk yield) were additive. An additive effect here implies that the effects of dietary FC ratio and SO supplement resulted in a combined effect that is almost equal to the sum of the individual effects. This is highlighted by the decrease in daily ruminal CH_4 emissions due the combined effect of FC ratio and SO supplement which corresponds closely to the sum of the individual effects. The decrease in daily ruminal CH_4 output of 13.5% when feeding H than L diets was associated with shifts in rumen fermentation toward propionate at the expense of acetate and a lower rumen pH. Such changes are consistent with less digestion of fiber fractions in animals fed H than L diet. Compared with fiber, fermentation of starch in the rumen may inhibit CH_4 formation by reducing rumen pH, ratio of acetogenic to gluconeogenic precursors in rumen VFA, and number and activity of rumen protozoa (Martin et al., 2010). Diets that promote the propionate synthesis in the rumen reduce the availability of hydrogen for CH_4 production by rumen methanogens (McAllister and Newbold, 2008). However, the proportionate decreases in CH_4 emissions with H diets (mean 13.4%) were about half of the decrease in the molar ratio of acetate and butyrate to propionate in rumen VFA (mean 22.3%). Changes in ruminal CH_4 emissions due to dietary FC ratio may be accompanied by alterations in rumen VFA profiles (Aguerre et al., 2011). However, the molar proportions of ruminal VFA are not a direct measure of VFA production but rather indicate the balance between production and absorption of VFA (Aguerre et al., 2011). When the effect of OM digested in the rumen is taken into account, the decrease in CH_4 emissions due to the high-concentrate diet was 34.3%. Overall, the effects of FC ratio on ruminal CH_4 emissions are consistent with, but greater than, the reports from lactating cows where forages were replaced by concentrates in the diet (Ferris et al., 1999; Aguerre et al., 2011).

The net impact of greater proportion of concentrates in the diet or lipid supplement on total farm CH_4 and GHG emissions are likely to be smaller than direct measurements of enteric emissions would suggest, because the decreases in total tract OM digestibility could be expected to increase CH_4 emissions from the manure (IPCC, 2006). Nevertheless, assessment of the impact of replacing forage with concentrate ingredients needs to account for GHG emissions from cereal compared with silage production (Lovett et al., 2006), the higher risk of ruminal acidosis (Nocek, 1997), and increased competition for human food sources before firm conclusions can be drawn on the sustainability of these approaches.

Dietary supplement of SO included in both L and H diets depressed daily ruminal CH_4 emissions by 22.2%, on average, due at least in part, to less extensive digestion of OM in the rumen (10.5%). The mitigating potential of SO on CH_4 emissions in the present study (mean response 4.5% per 10 g oil/kg diet DM) is marginally greater than 3.8% reported for a wide range of sources and amount of added lipid (Martin et al., 2010). Several mechanisms have been proposed to explain the influence of dietary lipid supplements on ruminal CH_4 production, including decreases in the amount of OM fermented in the rumen, adverse effects of 12:0 and 14:0 on ruminal protozoal populations (Hristov et al., 2011), and the inhibitory effects of unsaturated 18-carbon fatty acids on ruminal methanogens (Sousa et al., 2013). A shift in rumen fermentation from acetate and butyrate to propionate has been proposed as 1 explanation for a decrease in CH_4 production. However, in the present experiment, the decrease in CH_4 emissions per unit of OM digested in the rumen (14.8%) may not be solely explained by the marginal decrease in the molar acetate to propionate ratio in rumen VFA (1.4%) due to SO supplementation.

Dietary supplement of SO reduced CH_4 emissions per kg ECM by 23.3% in combination with the L diet, equivalent to an average response of 4.7% per 10 g oil/kg diet DM. Decreases in CH_4 emissions due to SO supplement were greater with L than H diet when expressed as g/kg OM intake or per unit total tract OM digestion. Literature reports on the simultaneous evaluation of FC ratio and oil supplementation on CH_4 emissions in cattle are scarce. In growing cattle, no interaction between dietary FC ratio and coconut oil supplementation was observed (Lovett et al., 2003). In lactating cows, the efficacy of linseed oil for reducing CH_4 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 CH_4 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). Nevertheless, data from the present experiment supports the concept that the effectiveness of lipid supplement for mitigating CH_4 emissions in lactating cows is dependent on the composition of the basal diet. Given that SO had less adverse effects on intake, digestion and milk fat yield when included in L than H diet (Ventto et al., 2017), it appears that the use of plant oils for reducing ruminal CH_4 emissions may be more effective in high-forage diets.

In general, strategies for mitigating enteric CH_4 emissions should also strive to avoid or possibly reduce emissions of non-enteric GHG (Martin et al., 2010). Both the greater proportion of concentrates and inclusion of SO supplement in diet decreased CH_4 emissions without increasing fecal and urinary N as a proportion of N intake which can be converted to ammonia or nitrous oxide under different manure storage conditions (IPCC, 2006). However, in our experiment non-enteric GHG emissions were not measured and thus evaluation of the possible trade-offs between reducing CH_4 emissions and non-enteric GHG emissions due to the dietary treatments was not possible.

In conclusion, the greater proportion of concentrates in the diet reduced daily ruminal CH_4 emissions which was associated with changes in rumen fermentation toward propionate, a lower ruminal pH and reduced fiber digestion. Dietary supplement of SO decreased daily ruminal CH_4 emissions that was explained, at least in part, by less extensive digestion of OM in the rumen. The effects of SO and greater proportion of concentrates in the diet on daily CH_4 emissions were additive but the additivity declined or vanished when different emission intensity indices were considered. Overall, SO was more effective in reducing CH_4 emissions when low-concentrate diet was fed.

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